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Specification and Drawings, as originally filed, with Application for Patent Serial No: **2,283,458,** on September 28, 1999, by **INFECTIO DIAGNOSTIC (I.D.I.) INC.,** assignee of Michel Bergeron , Maurice Boissinot, Ann Huletsky, Christian Ménard, Marc Ouellette, François J. Picard and Paul H. Roy, for "Highly Conserved Genes and Their Use to Generate Species-Specific, Genus-Specific, Family-Specific, Group-Specific and Universal Nucleic Acid Probes and Amplification Primers to Rapidly Detect and Identify Bacterial, Fungal and Parasitical Pathogens From Clinical Specimens for Diagnosis".

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ABSTRACT OF THE INVENTION

This invention relates to a repertory of nucleic sequences usable for th detection and/or identification of a bacterial, fungal or parasitical species, genus, family or group. This repertory is created by amplifying the nucleic acids of a plurality of given species with given primers. From this repertory are derived species-, genus-, family- or group-specific oligonucleotides used as probes or primers. Also, universal probes or primers are derived from the same repertory. All these probes or primers can be used in conjunction with probes or primers specific to the detection of any antibiotic resistance gene and/or toxin gene, in kits or methods designed for the detection of a set of bacteria, fungi or parasites, in association or not with antibiotic resistance or toxin production.

TITLE OF THE INVENTION

HIGHLY CONSERVED GENES AN THEIR USE TO GENERATE SPECIES-SPECIFIC, GENUS-SPECIFIC, FAMILY-SPECIFIC, GROUP-SPECIFIC AND UNIVERSAL NUCLEIC ACID PROBES AND AMPLIFICATION PRIMERS TO RAPIDLY DETECT AND IDENTIFY BACTERIAL, FUNGAL AND PARASITICAL PATHOGENS FROM CLINICAL SPECIMENS FOR DIAGNOSIS

BACKGROUND OF THE INVENTION

Classical methods for the identification of bacteria, fungi, and parasites

Bacteria and fungi are classically identified by their ability to utilize different substrates as a source of carbon and nitrogen through the use of biochemical tests such as the API20E™ system (bioMérieux). For susceptibility testing, clinical microbiology laboratories use methods including disk diffusion, agar dilution and broth microdilution. Although identifications based on biochemical tests and antibacterial susceptibility tests are cost-effective, at least two days are required to obtain preliminary results due to the necessity of two successive overnight incubations to identify the bacteria from clinical specimens as well as to determine their susceptibility to antimicrobial agents. There are some commercially available automated systems (i.e. the MicroScan™ system from Dade Diagnostics Corp. and the Vitek system from bioMérieux) which use sophisticated and expensive apparatus for faster microbial identification and susceptibility testing (Stager and Davis, 1992, Clin. Microbiol. Rev. 5:302-327). These systems require shorter incubation periods, thereby allowing most bacterial identifications and susceptibility testing to be performed in less than 6 hours. Nevertheless, these faster systems always require the primary isolation of the bacteria or fungi as a pure culture, a process which takes at least 18 hours for a pure culture or 2 days for a mixed culture. Moreover, fungi other than yeasts are often difficult or very slow to cultivate from clinical specimens. Identification must rely on labor intensive technique such as direct microscopic examination of the specimens and by direct and/or indirect immunological assays. Cultivation of most parasites is impractical in the clinical laboratory. Hence, microscopic examination of the specimen, a few immunological tests and clinical

symptoms are often the only methods used for an identification that frequently remains presumptive.

The fastest bacterial identification system, the autoSCAN-Walk-Away™ system (Dade Diagnostics Corp.) identifies both gram-negative and gram-positive bacterial species from standardized inoculum in as little as 2 hours and gives susceptibility patterns to most antibiotics in 5.5 hours. However, this system has a particularly high percentage (i.e. 3.3 to 40.5%) of non-conclusive identifications with bacterial species other than *Enterobacteriaceae* (Croizé J., 1995, Lett. Infectiol. **10**:109-113; York *et al.*, 1992, J. Clin. Microbiol. **30**:2903-2910). For *Enterobacteriaceae*, the percentage of non-conclusive identifications was 2.7 to 11.4%.

A wide variety of bacteria and fungi are routinely isolated and identified from clinical specimens in microbiology laboratories. Tables 1 and 2 give the incidence for the most commonly isolated bacterial and fungal pathogens from various types of clinical specimens. These pathogens are the main organisms associated with nosocomial and community-acquired human infections and are therefore considered the most clinically important.

Clinical specimens tested in clinical microbiology laboratories

Most clinical specimens received in clinical microbiology laboratories are urine and blood samples. At the microbiology laboratory of the Centre Hospitalier de l'Université Laval (CHUL), urine and blood account for approximately 55% and 30% of the specimens received, respectively (Table 3). The remaining 15% of clinical specimens comprise various biological fluids including sputum, pus, cerebrospinal fluid, synovial fluid, and others (Table 3). Infections of the urinary tract, the respiratory tract and the bloodstream are usually of bacterial etiology and require antimicrobial therapy. In fact, all clinical samples received in the clinical microbiology laboratory are tested routinely for the identification of bacteria and susceptibility testing.

Conventional pathogen identification from clinical specimens

Urine specimens

The search for pathogens in urine specimens is so preponderant in the routine microbiology laboratory that a myriad of tests have been developed. However, the gold standard remains the classical semi-quantitative plate culture method in which 1 µL of urine is streaked on plates and incubated for 18-24 hours. Colonies are then counted to determine the total number of colony forming units (CFU) per liter of urine. A bacterial urinary tract infection (UTI) is normally associated with a bacterial

count of 10^7 CFU/L or more in urine. However, infections with less than 10^7CFU/L in urine are possible, particularly in patients with a high incidence of diseases or those catheterized (Stark and Maki, 1984, N. Engl. J. Med. **311**:560-564). Importantly, approximately 80% of urine specimens tested in clinical microbiology laboratories are considered negative (i.e. bacterial count of less than 10^7 CFU/L; Table 3). Urine specimens found positive by culture are further characterized using standard biochemical tests to identify the bacterial pathogen and are also tested for susceptibility to antibiotics. The biochemical and susceptibility testing normally require 18-24 hours of incubation.

Accurate and rapid urine screening methods for bacterial pathogens would allow a faster identification of negative specimens and a more efficient treatment and care management of patients. Several rapid identification methods (Uriscreen™, UTIscreen™, Flash Track™ DNA probes and others) have been compared to slower standard biochemical methods, which are based on culture of the bacterial pathogens. Although much faster, these rapid tests showed low sensitivities and poor specificities as well as a high number of false negative and false positive results (Koening *et al.*, 1992, J. Clin. Microbiol. **30**:342-345; Pezzlo *et al.*, 1992, J. Clin. Microbiol. **30**:640-684).

Blood specimens

The blood specimens received in the microbiology laboratory are always submitted for culture. Blood culture systems may be manual, semi-automated or completely automated. The BACTEC system (from Becton Dickinson) and the BacTAlert system (from Organon Teknika Corporation) are the two most widely used automated blood culture systems. These systems incubate blood culture bottles under optimal conditions for bacterial growth. Bacterial growth is monitored continuously to detect early positives by using highly sensitive bacterial growth detectors. Once growth is detected, a Gram stain is performed directly from the blood culture and then used to inoculate nutrient agar plates. Subsequently, bacterial identification and susceptibility testing are carried out from isolated bacterial colonies with automated systems as described previously. The bottles are normally reported as negative if no growth is detected after an incubation of 6 to 7 days. Normally, the vast majority of blood cultures are reported negative. For example, the percentage of negative blood cultures at the microbiology laboratory of the CHUL for the period February 1994-January 1995 was 93.1% (Table 3).

Other clinical samples

Upon receipt by the clinical microbiology laboratory, all body fluids other than blood and urine that are from normally sterile sites (i.e. cerebrospinal, synovial,

pleural, pericardial and others) are processed for direct microscopic examination and subsequent culture. Again, most clinical samples are negative for culture (Table 3). In these normally sterile site, a test for the universal detection of bacteria, fungi and parasites would be very useful.

Regarding clinical specimens which are not from sterile sites such as sputum or stool specimens, the laboratory diagnosis by culture is more problematic because of the contamination by the normal flora. The bacterial or fungal pathogens potentially associated with the infection are purified from the contaminants and then identified as described previously. Of course, the universal detection of bacteria would not be useful for the diagnosis of bacterial infections at these non sterile sites. On the other hand, DNA-based assays for species or genus or family or group detection and identification as well as for the detection of antibiotic resistance genes from these specimens would be very useful and would offer several advantages over classical identification and susceptibility testing methods.

DNA-based assays with any specimens

There is an obvious need for rapid and accurate diagnostic tests for the detection and identification of bacteria, fungi and parasites directly from clinical specimens. DNA-based technologies are rapid and accurate and offer a great potential to improve the diagnosis of infectious diseases (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). The DNA probes and amplification primers which are objects of the present invention are applicable for the detection and identification of bacteria, fungi, and parasites directly from any clinical specimens such as blood cultures, blood, urine, sputum, cerebrospinal fluid, pus and other type of specimens (Table 3). The DNA-based tests proposed in this invention are superior in terms of both rapidity and accuracy to standard biochemical methods currently used for routine diagnosis from any clinical specimens in microbiology laboratories. Since these tests can be performed in one hour or less, they provide the clinicians with new diagnostic tools which should contribute to increase the efficiency of therapies with antimicrobial agents. Specimens from sources other than humans (e.g. other primates, birds, plants, mammals, farm animals, livestock, food products, water and others) may also be tested with these assays.

A high percentage of culture negative specimens

Among all the clinical specimens received for routine diagnosis, approximately 80% of urine specimens and even more (around 95%) for other types of clinical specimens are negative for the presence of bacterial pathogens (Table 3). It would

also be desirable, in addition to identify bacteria at the species or genus or family or group level in a given specimen, to screen out the high proportion of negative clinical specimens with a test detecting the presence of any bacterium (i.e. universal bacterial detection). Such a screening test may be based on DNA amplification by PCR of a highly conserved genetic target found in all bacteria. Specimens negative for bacteria would not be amplified by this assay. On the other hand, those that are positive for bacteria would give a positive amplification signal with this assay. Similarly, highly conserved genes of fungi and parasites could serve not only to identify particular species or genus or family or group but also to detect the presence of any fungi or parasite in the specimen.

Towards the development of rapid DNA-based diagnostic tests

A rapid diagnostic test should have a significant impact on the management of infections. DNA probe and DNA amplification technologies offer several advantages over conventional methods for the identification of pathogens and antibiotic resistance genes from clinical samples (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.; Ehrlich and Greenberg, 1994, PCR-based Diagnostics in Infectious Disease, Blackwell Scientific Publications, Boston, MA). There is no need for culture of the pathogens, hence the organisms can be detected directly from clinical samples, thereby reducing the time associated with the isolation and identification of pathogens. Furthermore, DNA-based assays are more accurate for microbial identification than currently used phenotypic identification systems which are based on biochemical tests and/or microscopic examination. Commercially available DNA-based technologies are currently used in clinical microbiology laboratories, mainly for the detection and identification of fastidious bacterial pathogens such as *Mycobacterium tuberculosis*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae* as well as for the detection of a variety of viruses (Podzorski and Persing, Molecular detection and identification of microorganisms,*In*: P. Murray *et al.*, 1995, Manual of Clinical Microbiology, ASM press, Washington D.C.). There are also other commercially available DNA-based assays which are used for culture confirmation assays.

Others have developed DNA-based tests for the detection and identification of bacterial pathogens which are objects of the present invention for example: *Staphylococcus* spp. (US patent application serial No. US 5 437 978), *Neisseria* spp. (US patent application serial No. US 5 162 199 and European patent application serial No. EP 0 337 896 131) and *Listeria monocytogenes* (US patent applications serial Nos US 5 389 513 and US 5 089 386). However, the diagnostic tests described in these patents are based either on rRNA genes or on genetic targets different from

those described in the present invention. To our knowledge there are only three patents published by others mentioning the use of any of the three targets described in the present invention for diagnostic purposes (PCT international publication number WO 92/03455, European patent publication number 0 133 671 B1, and European patent publication number 0 133 288 A2). WO 92/03455 is focused on the inhibition of *Candida* species for therapeutic purposes. It describes antisense oligonucleotide probes hybridizing to *Candida* messenger RNA. Two of the numerous mRNA proposed as target are coding for translation elongation factor 1 (tef1) and the beta subunit of ATPase. DNA amplification or hybrization are not under the scope of their invention and although diagnostic use is briefly mentioned in the body of the application, no specific claim are made regarding diagnostics. In the main body of the text, EP 0 133 671 B1 describes the use of bacterial *tuf* sequence for diagnostics based on hybridization with bacterial RNA. To hybridize RNA, an oligonucleotide probe must be antisense. DNA amplification techniques require the use of both sense and antisense primers. Hence, claim number one from EP 0 133 671 B1 precludes PCR or other DNA-based amplification techniques. Furthermore, EP 0 133 671 B1 makes no specific claim on the use of *tuf* sequences for diagnostics; only ribosomal RNA sequences are claimed. Patent EP 0 133 288 A2 describes and claims the use of bacterial *tuf* sequence for diagnostics based on hybridization of a *tuf* probe with bacterial DNA. DNA amplification is not in the scope of EP 0 133 288 A2. Nowhere; it is mentioned that multiple *tuf* probes could be used simultaneously. The sensitivity of the *tuf* hybrizations reported are, at $1x10^6$ bacteria or 1-100 ng of DNA, much lower than those achievable by nucleic acid amplification technologies.

Although there are diagnostic kits or methods already used in clinical microbiology laboratories, there is still a need for an advantageous alternative to the conventional identification methods in order to improve the accuracy and the speed of the diagnosis of commonly encountered bacterial infections. Besides being much faster, DNA-based diagnostic tests are more accurate than standard biochemical tests presently used for diagnosis because the microbial genotype (e.g. DNA level) is more stable than the phenotype (e.g. physiologic level).

Knowledge of the genomic sequences of bacterial, fungal and parasitical species continuously increases as testified by the number of sequences available from public databases such as GenBank. From the sequences readily available from those public databases, there is no indication therefrom as to their potential for diagnostic purposes. For determining good candidates for diagnostic purposes, one could select sequences for DNA-based assays for (i) the species-specific detection and identification of commonly encountered bacterial, fungal and parasitical pathogens, (ii) the genus-specific detection and identification of commonly encountered bacterial, fungal or parasitical pathogens, (iii) the universal detection of bacterial, fungal or parasitical pathogens and/or (iv) the specific detection and identification of

antibiotic resistance genes. All of the above types of DNA-based assays may be performed directly from any type of clinical specimens or from a microbial culture.

In our co-pending U.S. (N.S. 08/526,840) and PCT (PCT/CA/95/00528 and PCT/CA97/00829) patent applications, we described DNA sequences suitable for (i) the species-specific detection and identification of clinically important bacterial pathogens, (ii) the universal detection of bacteria, and (iii) the detection of antibiotic resistance genes.

The latter co-pending application described proprietary *tuf* DNA sequences as well as *tuf* sequences selected from public databases (in both cases, fragments of at least 100 base pairs), as well as oligonucleotide probes and amplification primers derived from these sequences. All the nucleic acid sequences described in that patent application can enter in the composition of diagnostic kits or product and methods capable of a) detecting the presence of bacteria, fungi and parasites b) detecting specifically at the species, genus, family or group levels, the presence of bacteria, fungi and parasites and antibiotic resistance genes associated with these pathogens. However, these methods and kits need to be improved, since the ideal kit and method should be capable of diagnosing close to 100% of microbial pathogens and associated antibiotic resistance genes and toxins genes. For example, infections caused by *Enterococcus faecium* have become a clinical problem because of its resistance to many antibiotics. Both the detection of these bacteria and the evaluation of their resistance profiles are desirable. Besides that, novel DNA sequences (probes and primers) capable of recognizing the same and other microbial pathogens or the same and additional antibiotic resistance genes are also desirable to aim at detecting more target genes and complement our earlier patent applications.

The present invention improves the co-pending application by disclosing new proprietary *tuf* sequences as well as describing new ways to abtain *tuf* sequences. In addition we disclose new proprietary *atpD* and *recA* sequences. In addition, new uses of *tuf*, *atpD* and *recA* DNA sequences selected from public databases are disclosed.

Highly conserved genes for identification and diagnostics

Highly conserved genes are useful for identification of microorganisms. For bacteria, the most studied genes for identification of microorganisms are the universally conserved ribosomal RNA genes (rRNA). Among those, the principal targets used for identification purposes are the small subunit (SSU) ribosomal 16S rRNA genes (in prokaryotes) and 18S rRNA genes (in eukaryotes) (Relman and Persing, Genotyping Methods for Microbial Identification, *In*: D.H. Persing, 1996, PCR Protocols for Emerging Infectious Diseases, ASM Press, Washington D.C.). The rRNA genes are also the most commonly used targets for universal identification of bacteria (Chen *et al.*, 1988, FEMS Microbiol. Lett. **57**:19-24; McCabe *et al.*, 1999, Mol. Genet. Metabol. **66**:205-211) and fungi (Van Burik *et al.*, 1998, J. Clin. Microbiol. **36**:1169-1175).

However, it may be difficult to discriminate between closely related species when using primers derived from the 16S rRNA. In some instances, 16S rRNA

sequence identity may not be sufficient to guarantee species identity (Fox *et al.*, 1992, Int. J. Syst. Bacteriol. 42:166-170) and it has been shown that inter-operon sequence variation as well as strain to strain variation could undermine the application of 16S rRNA for identification purposes (Clayton *et al.*, 1995, Int. J. Syst. Bacteriol. 45:595-599).

STATEMENT OF THE INVENTION

It is an object of the present invention to provide a specific, ubiquitous and sensitive method using probes and/or amplification primers for determining the presence and/or amount of nucleic acids:

- from any bacterial, fungal or parasitical species

in any sample suspected of containing said nucleic acids, and optionally,

- from specific microbial species or genera selected from the group consisting of the species or genera listed in Table 4
- from an antibiotic resistance gene selected from the group consisting of the genes listed in Table 5, and optionally,
- from a toxin gene selected from the group consisting of the genes listed in Table 6,

wherein each of said nucleic acids or a variant or part thereof comprises a selected target region hybridizable with said probe or primers;

said method comprising the steps of contacting said sample with said probes or primers and detecting the presence and/or amount of hybridized probes or amplified products as an indication of the presence and/or amount of said any microbial species, specific microbial species or genus or family or group and antibiotic resistance gene and/or toxin gene.

In a specific embodiment, a similar method directed to each specific microbial species or genus or family or group detection and identification, antibiotic resistance genes detection, toxin genes detection, and universal bacterial detection, separately, is provided.

In a more specific embodiment, the method makes use of DNA fragments from conserved genes (proprietary sequences and sequences obtained from public databases), selected for their capacity to sensitively, specifically and ubiquitously detect the targeted bacterial, fungal or parasitical nucleic acids.

In a particularly preferred embodiment, oligonucleotides of at least 12 nucleotides in length have been derived from the longer DNA fragments, and are used in the present method as probes or amplification primers.

In another particularly preferred embodiment, oligonucleotides primers and probes of at least 12 nucleotides in length are designed for their specificity and

ubiquity based upon analysis of our databases of*atpD*, *tuf* and *recA* sequences. These databases are generated using both proprietary and public databases sequence information. Altogether, these databases form a sequence repertory useful for the design of primers and probes for the detection and identification of bacterial, fungal and parasitical microorganisms. The repertory can also be subdivided into subrepertories for analysis leading to the design of primers and probes.

The *atpD*, *tuf* and *recA* sequences databases as a product to assist the design of oligonucleotides primers and probes for the detection and identification of bacterial, fungal and parasitical microorganisms are also an object of this invention.

The proprietary oligonucleotides (probes and primers) are also another object of the invention.

Diagnostic kits comprising probes or amplification primers for the detection of a microbial species or genus or family or group selected from the following list consisting of *Bordetella* spp., *Candida albicans, Candida dubliniensis, Candida* spp., *Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium* spp., *Corynebacterium* spp., *Cryptosporidium parvum, Entamoeba* spp., *Enterobacteriaceae* group, *Enterococcus casseliflavus-flavescens-gallinarum, Enterococcus faecalis, Enterococcus faecium, Enterococcus gallinarum, Enterococcus* spp., *Escherichia coli, Giardia* spp., *Haemophilus influenzae, Kinetoplastidae* group, *Leishmania* spp., *Mycobacteriaceae* family, *Neisseria gonorrhoeae,* platelets contaminants group, *Pseudomonads group, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus saprophyticus, Staphylococcus* spp., *Streptococcus agalactiae, Streptococcus* spp., *Trypanosoma brucei, Trypanosoma cruzi, Trypanosoma*spp., are also objects of the present invention.

Diagnostic kits further comprising probes or amplification primers for the detection of an antibiotic resistance gene selected from the group listed in Table 5 are also objects of this invention.

Diagnostic kits further comprising probes or amplification primers for the detection of a toxin gene selected from the group listed in Table 6 are also objects of this invention.

Diagnostic kits further comprising probes or amplification primers for the detection of any bacterial, fungal or parasitical species, comprising or not comprising those for the detection of the specific microbial species or genus or family or group listed above, and further comprising or not comprising probes and primers for the antibiotic resistance genes listed above, and further comprising or not comprising probes and primers for the toxin genes listed above are also objects of this invention.

In a preferred embodiment, such a kit allows for the separate or the simultaneous detection and identification of the above-listed microbial species or

genus or family or group, antibiotic resistance genes, toxin genes and for the detection of any microorganism (bacteria, fungi or parasite).

In the above methods and kits, amplification reactions may include but are not restricted to: a) polymerase chain reaction (PCR), b) ligase chain reaction (LCR), c) nucleic acid sequence-based amplification (NASBA), d) self-sustained sequence replication (3SR), e) strand displacement amplification (SDA), f) branched DNA signal amplification (bDNA), g) transcription-mediated amplification (TMA), h) cycling probe technology (CPT), i) nested PCR, j) multiplex PCR, k) solid phase amplification (SPA), l) nuclease dependant signal amplification (NDSA).

In a preferred embodiment, a PCR protocol is used for nucleic acid amplification.

In a particularly preferred embodiment, a PCR protocol is provided, comprising, an initial denaturation step of 1-3 minutes at 95°C, followed by amplification cycle including a denaturation step of one seconds at 95°C and an annealing step of 30 seconds at 45-65°C, without any time allowed specifically for the elongation step. This PCR protocol has been standardized to be suitable for PCR reactions with most selected primer pairs, which greatly facilitates the testing because each clinical sample can be tested with universal, species-specific, genus-specific and antibiotic resistance gene PCR primers under uniform cycling conditions. Furthermore, various combinations of primer pairs may be used in multiplex PCR assays.

We aim at developing a rapid test or kit to discard rapidly all the samples which are negative for bacterial cells and to subsequently detect and identify the above bacterial and/or fungal and/or parasitical species and genera and to determine rapidly the bacterial resistance to antibiotics. Although the sequences from the selected antibiotic resistance genes are available from public databases and have been used to develop DNA-based tests for their detection, our approach is unique because it represents a major improvement over current gold standard diagnostic methods based on bacterial cultures. Using an amplification method for the simultaneous bacterial detection and identification and antibiotic resistance genes detection, there is no need for culturing the clinical sample prior to testing. Moreover, a modified PCR protocol has been developed to detect all target DNA sequences in approximately one hour under uniform amplification conditions. This procedure will save lives by optimizing treatment, will diminish antibiotic resistance because less antibiotics will be prescribed, will reduce the use of broad spectrum antibiotics which are expensive, decrease overall health care costs by preventing or shortening hospitalizations, and decrease the time and costs associated with clinical laboratory testing.

In the methods and kits described herein below, the oligonucleotide probes and amplification primers have been derived from larger sequences (i.e. DNA fragments of at least 100 base pairs). All DNA fragments have been obtained either from

proprietary fragments or from public databases. DNA fragments selected from public databases are newly used in a method of detection according to the present invention, since they have been selected for their diagnostic potential.

In an other embodiment, the amino acid sequences translated from the repertory of *atpD*, *tuf* and *recA* sequences are also an object of the present invention.

It is clear to the individual skilled in the art that other oligonucleotide sequences appropriate for (i) the universal bacterial detection, (ii) the detection and identification of the above microbial species or genus or family or group, and (iii) the detection of antibiotic resistance genes, and (iv) the detection of toxin genes other than those listed in Annexes I to III and XXI may also be derived from the proprietary fragments or selected public databases sequences. For example, the oligonucleotide primers or probes may be shorter or longer than the ones we have chosen; they may also be selected anywhere else in the proprietary DNA fragments or in the sequences selected from public databases; they may be also variants of the same oligonucleotide. If the target DNA or a variant thereof hybridizes to a given oligonucleotide, or if the target DNA or a variant thereof can be amplified by a given oligonucleotide PCR primer pair, the converse is also true; a given target DNA may hybridize to a variant oligonucleotide probe or be amplified by a variant oligonucleotide PCR primer. Alternatively, the oligonucleotides may be designed from any DNA fragment sequences for use in amplification methods other than PCR. Consequently, the core of this invention is the identification of universal, species-specific, genus-specific, resistance gene-specific, toxin gene-specific genomic or non-genomic DNA fragments which are used as a source of specific and ubiquitous oligonucleotide probes and/or amplification primers. Although the selection and evaluation of oligonucleotides suitable for diagnostic purposes requires much effort, it is quite possible for the individual skilled in the art to derive, from the selected DNA fragments, oligonucleotides other than the ones listed in Annexes I to III and XXI which are suitable for diagnostic purposes. When a proprietary fragment or a public databases sequence is selected for its specificity and ubiquity, it increases the probability that subsets thereof will also be specific and ubiquitous.

Since a high percentage of clinical specimens are negative for bacteria (Table 3), DNA fragments having a high potential for the selection of universal oligonucleotide probes or primers were selected from proprietary and public databases sequences. The amplification primers were selected from two genes highly conserved in bacteria, fungi and parasites, and are used to detect the presence of any bacterial or fungal or parasitical pathogen in clinical specimens in order to determine rapidly (less than one hour) whether it is positive or negative for bacteria, fungi or parasites. The selected genes, designated *tuf, atpD* and *recA,* encode respectively a protein (elongation factor Tu) involved in the translational process during protein synthesis, a protein (beta subunit) responsible for the catalytic activity of proton

pump ATPase and a protein responsible for the homologous recombination of genetic material. The *tuf, atpD* and *recA* sequence alignments used to derive the universal primers include both proprietary and public databases sequences. The universal primer strategy allows the rapid screening of the numerous negative clinical specimens (around 80% of the specimens received, see Table 3) submitted for bacteriological testing.

Table 4 provides a list of the bacterial, fungal and parasitical species for which *atpD* and/or *tuf* and/or *recA* sequences are revealed in the present invention. Tables 5 and 6 provide a list of antibiotics resistance genes and toxin genes selected for diagnostic purposes. Table 7 provides the origin of *tuf, atp*D and *rec*A sequences listed in the sequence listing. Tables 8-10 provide lists or species used to test specificity and ubiquity of some assays described in examples.

DETAILED DESCRIPTION OF THE INVENTION

HIGHLY CONSERVED GENES AND THEIR USE TO GENERATE SPECIES-SPECIFIC, GENUS-SPECIFIC, FAMILY-SPECIFIC, GROUP-SPECIFIC AND UNIVERSAL NUCLEIC ACID PROBES AND AMPLIFICATION PRIMERS TO RAPIDLY DETECT AND IDENTIFY BACTERIAL, FUNGAL AND PARASITICAL PATHOGENS FROM CLINICAL SPECIMENS FOR DIAGNOSIS

The present inventors compared the published *Haemophilus influenzae* and *Mycoplasma genitalium* genomes and searched for the most conserved genes, which would then serve, as paradigm to develop primers and probes. This sequence comparison is highly informative as these two bacteria are distantly related and most genes present in the minimal genome of *M. genitalium* are likely to be present in every bacterium. Therefore genes conserved between these two bacteria are likely to be conserved in all other bacteria.

Following the genomic comparison, it was found that several protein coding genes were conserved in evolution. Highly conserved proteins included the translation elongation factor Tu (EF-Tu) and the β subunit of F_0F_1 type ATP-synthase, and to a lesser extend, the RecA recombinase.

Translation elongation factor Tu is a member of a family of GTP-binding proteins which intervene in the interactions of tRNA molecules with the ribosome machinery during essential steps of protein synthesis. The role of elongation factor Tu is to facilitate the binding of aminoacylated tRNA molecules to the A site of the ribosome. The eukaryotic and archaebacterial homolog of EF-Tu is called elongation factor 1 alpha (EF-1α). All protein synthesis factors originated from a common ancestor via gene duplications and fusions (Cousineau *et al.*, 1997, J. Mol. Evol. **45**:661-670). In addition, EF-Tu is known to be the target for antibiotics belonging to the elfamycin's group as well as to other structural classes (Anborgh and Parmeggiani, 1991, EMBO J. **10**:779-784; Luiten *et al.*, 1992, European patent application serial No. EP 0 466 251 A1). Interestingly, a form of the EF-Tu protein has been identified as a dominant component of the periplasm of *Neisseria gonorrhoeae* (Porcella *et al.*, 1996, Microbiology **142**:2481-2489), hence suggesting that at least in some bacterial species, EF-Tu might be an antigen with vaccine potential.

F_0F_1 type ATP-synthase belongs to a superfamily of proton-translocating ATPases divided in three major families: P, V and F (Nelson and Taiz, 1989, TIBS **14**:113-116). P-ATPases (or E_1-E_2 type) operate via a phosphorylated intermediate and are not evolutionarily related to the other two families. V-ATPases (or V_0V_1 type) are present on the vacuolar and other endomembranes of eukaryotes, on the plasma membrane of archaebacteria and also on the plasma membrane of some eubacteria especially, species belonging to the order *Spirochaetales* as well as to the *Chlamydiaceae* and *Deinococcaceae* families. F-ATPases (or F_0F_1 type) are found on the plasma membrane of most eubacteria, on the inner membrane of mitochondria

and on the thylakoid membrane of chloroplasts. They function mainly in ATP synthesis. They are large multimeric enzymes sharing numerous structural and functional features with the V-ATPases. F and V-type ATPases have diverged from a common ancestor in an event preceding the appearance of eukaryotes. The β subunit of the F-ATPases is the catalytic subunit and it possess low but significant sequence homologies with the catalytic A subunit of V-ATPases.

The translation elongation factor Tu (EF-Tu or EF-1α) and the catalytic subunit of F or V-types ATP-synthase are two highly conserved proteins sometimes used for phylogenetic analysis and their genes are also known to be highly conserved (Iwabe *et al.*, 1989, Proc. Natl. Acad. Sci. USA **86**:9355-9359, Gogarten *et al.*, 1989, Proc. Natl. Acad. Sci. USA **86**:6661-6665, Ludwig *et al.*, 1993, Antonie van Leeuwenhoek **64**:285-305). A recent BLAST (Altschul *et al.*, 1997, J. Mol. Biol. **215**:403-410) search performed by the present inventors on the GenBank, EMBL, DDBJ and specific genome project databases indicated that throughout bacteria, the EF-Tu and the β subunit of F_0F_1 type ATP-synthase genes may be more conserved than other genes that are well conserved between *H. influenzae* and *M. genitalium*.

The RecA recombinase is a multifunctional protein encoded by the *recA* gene. It plays a central role in homologous recombination, it is critical for the repair of DNA damage and it is involved in the regulation of the SOS system by promoting the proteolytic digestion the LexA repressor. It is highly conserved in bacteria and could serve as a useful genetic marker to reconstruct bacterial phylogeny (Miller and Kokjohn, 1990, Annu. Rev. Microbiol. **44**:365-394). Althought *recA* possess some highly conserved sequence segments that we used to design universal primers aimed at sequencing the *recA* fragments, it is clearly not as well conserved as *tuf* and *atpD*. Hence, *recA* may not be optimal for universal detection of bacteria with high sensitivity but it was chosen as preliminary data indicated that *tuf* and *atpD* may sometimes be too closely related to find specific primer pairs that could discriminate between certain very closely related species and genera. While RecA, like *tuf* and *atp*D, possess highly conserved regions suitable for the design of universal sequencing primers, the less conserved region between primers should be divergent enough to allow species-specific and genus-specific primers in those cases.

Thus, as targets to design primers and probes for the genetic detection of microorganisms, the present inventors have concentrated on the genes encoding these three proteins: *tuf*, the gene for elongation factor Tu; and *atpD*, the gene for β subunit of F_0F_1 type ATP-synthase; and *recA* the gene encoding the RecA recombinase. In several bacterial genomes *tuf* is often found in two highly similar duplicated copies named *tufA* and *tufB* (Filer and Furano, 1981, J. Bacteriol. **148**:1006-1011, Sela *et al.*, 1989, J. Bacteriol. **171**:581-584). In some particular cases, more divergent copies of the *tuf* genes can exist in some bacterial species such as some Actinomycetes (Luiten *et al.* European patent application publication No. EP 0 446 251 A1; Vijgenboom *et al.*, 1994, Microbiology **140**:983-998) and, as revealed as part of this invention, in several enterococcal species. The *tuf*, *atpD* and *recA* genes were chosen as there are well conserved in evolution and have highly conserved stretches as well as more variable segments. Moreover, these three genes have eukaryotic orthologs which are

described in the present invention as targets to identify fungi and parasites. The eukaryotic homolog of elongation factor Tu is called elongation factor 1-alpha (EF-1α) (gene name: *tef, tef1, ef1, ef-1* or *EF-1*). In fungi, the gene for EF-1α occurs sometimes in two or more highly similar duplicated copies (often named *tef1, tef2, tef3*...). In addition, eukaryotes have a copy of elongation factor Tu which is originating from their organelle genome ancestery (gene name: *tuf1* or *tufM*). For the purpose of the current invention, the genes of these three (bacterial, eukaryotic and organellar) functionally and evolutionarily related elongation factors will hereafter be designated as «*tuf* sequences». The eukaryotic (mitochondrial) F_0F_1 type ATP-synthase beta subunit gene is named *atp2* in yeast. For the purpose of the current invention, the genes of catalytic subunit of either F or V-type ATP-synthase will hereafter be designated as «*atpD* sequences». The eukaryotic homologs of RecA are distributed in two families, typified by the Rad51 and Dmc1 proteins. For the purpose of the current invention, the genes corresponding to the latter proteins will hereafter be designated as «*recA* sequences».

Analysis of multiple sequence alignments of *tuf* and *atp*D sequences present in the public databases, permitted the design of oligonucleotide primers (and probes) capable of amplifying (or hybridizing to) segments of *tuf* and *atp*D genes from a wide variety of bacterial species (see Examples 1 to 4 and Table 7). Sequencing primer pairs for *tuf* sequences are listed in Annex I and hybridization probes are listed in Annex III. Sequencing primer pairs for *atpD* sequences are listed in Annex II. Analysis of the main subdivisions of *tuf* and *atpD* sequences (see Figures 1 and 2) permitted to design sequencing primers amplifying specifically each of these subdivisions. It should be noted that these sequencing primers could also be use as universal primers. However, since some of these sequencing primers include several variable sequence (degenerated) positions, their sensitivity could be lower than that of universal primers developed for diagnostic purposes. Further subdivisions could be done on the basis of the various phyla where these genes are encountered.

Similarly, analysis of multiple sequence alignments of *recA* sequences present in the public databases, permitted the design of oligonucleotide primers capable of amplifying segments of *recA* genes from a wide variety of bacterial species. Sequencing primer pairs for *recA* sequences are listed in Annex XXI. The main subdivisions of *recA* sequences comprise *recA*, *rad51* and *dmc1*. Further subdivisions could be done on the basis of the various phyla where these genes are encountered.

The present inventor's strategy is to get as much sequence data information from the three conserved genes (*tuf, atpD* and *recA*). This ensemble of sequence data forming a repertory (with subrepertories corresponding to each target genes and their main sequence subdivisions) and then using the sequence information of the sequence repertory (or subrepertories) to design primer pairs that could permit either universal detection of bacteria or fungi or parasites, detection of a family or group of microorganism (e.g. *Enterobacteriaceae*), detection of a genus (e.g. *Streptococcus*) or finally a specific species (e.g. *Staphylococcus aureus*). It should be noted that for the purpose of the present invention a group of microorganisms is defined depending on the needs of the particular diagnostic test. It does not need to respect a particular taxonomical grouping or phylum. See example 12 where primers were designed to

amplify a group a bacteria consisting of the 17 bacterial species most frequently encountered as platelet contaminants. Also remark that in that example, the primers' specificity is not perfect since the objective of that particular test is to be able to sensitively and rapidly detect at least the 17 most frequently encountered species but, the primers could also detect other species as well. In these circonstances the primers shown in example 12 are considered universal for platelet-contaminating bacteria. To develop an assay specific for the latter, one or more primers or probes specific to each species could be design. Another example of primers and/or probes for group detection is given by the Pseudomonad group primers. These primers were designed based upon alignment of *tuf* sequences from real *Pseudomonas* species as well as from former *Pseudomonas* species such as *Stenotrophomonas maltophilia*. The resulting primers are able to amplify all *Pseudomonas* species tested as well as several species belonging to different genera, hence we as beeing specific for a group including *Pseudomonas* and other species, we defined that group as*Pseudomonas* as several members were former *Pseudomonas*.

For certain applications, it may be possible to develop a universal, group, family or genus-specific reaction and to proceed to species identification using sequence information within the amplicon to design species-specific internal probes or primers, or alternatively, to proceed directly by sequencing the amplicon. The various strategies will be discussed further below.

The ensembles formed by public and proprietary *tuf, atpD* and *rec*A sequences are used in a novel fashion so they constitute three databases containing useful information for the identification of microorganisms.

Oligonucleotide primers and probes design and synthesis

The *tuf, atpD* and *recA* sequences DNA fragments sequenced by us or selected from public databases (GenBank and EMBL) were used to design oligonucleotides primers and probes for diagnostic purposes. We also relied on the corresponding peptide sequence of *tuf, atpD* and *recA* sequences to facilitate the identification of regions suitable for primers and probes design. As part of the design rules, all oligonucleotides (probes for hybridization and primers for DNA amplification) were evaluated for their suitability for hybridization or DNA amplification by polymerase chain reaction (PCR) by computer analysis using standard programs (i.e. the Genetics Computer Group (GCG) programs and the primer analysis software Oligo™ 5.0). The potential suitability of the PCR primer pairs was also evaluated prior to the synthesis by verifying the absence of unwanted features such as long stretches of one nucleotide and a high proportion of G or C residues at the 3' end (Persing*et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). Oligonucleotide probes and amplification primers were synthesized using an automated DNA synthesizer (Perkin-Elmer Corp., Applied Biosystems Division).

The oligonucleotide primers or probes may be derived from either strand of the duplex DNA. The primers or probes may consist of the bases A, G, C, or T or analogs and they may be degenerated at one or more chosen nucleotide position(s). The primers or probes may be of any suitable length and may be selected anywhere within the DNA sequences from proprietary fragments or from selected database sequences which are suitable for (i) the universal detection of bacteria or fungi or parasites, (ii) the species-specific detection and identification of *Candida albicans, Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Cryptosporidium parvum, Enterococcus faecalis, Enterococcus faecium, Enterococcus gallinarum, Escherichia coli, Haemophilus influenzae, Neisseria gonorrhoeae, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus saprophyticus, Streptococcus agalactiae, Trypanosoma brucei, Trypanosoma cruzi,* (iii) the genus-specific detection of *Bordetella* species, *Candida* species, *Clostridium* species, *Corynebacterium* species, *Entamoeba* species, *Enterococcus* species, *Giardia* species, *Leishmania* species, *Staphylococcus* species, *Streptococcus* species, *Trypanosoma* species, (iv) the family-specific detection of *Enterobacteriaceae* family members, *Mycobacteriaceae* family members, (v) the detection of *Enterococcus casseliflavus-flavescens-gallinarum* group, *Kinetoplastidae* group, *Pseudomonas* extended group, Platelet contaminating bacteria group, or (vi) the detection of clinically important antibiotic resistance genes listed in Table 5, or (vii) the detection of clinically important toxin genes listed in Table 6.

Variants for a given target bacterial gene are naturally occurring and are attributable to sequence variation within that gene during evolution (Watson *et al.*, 1987, Molecular Biology of the Gene, 4th ed., The Benjamin/Cummings Publishing Company, Menlo Park, CA; Lewin, 1989, Genes IV, John Wiley & Sons, New York, NY). For example, different strains of the same bacterial species may have a single or more nucleotide variation(s) at the oligonucleotide hybridization site. The person skilled in the art is well aware of the existence of variant bacterial or fungal DNA sequences for a specific gene and that the frequency of sequence variations depends on the selective pressure during evolution on a given gene product. The detection of a variant sequence for a region between two PCR primers may be demonstrated by sequencing the amplification product. In order to show the presence of sequence variants at the primer hybridization site, one has to amplify a larger DNA target with PCR primers outside that hybridization site. Sequencing of this larger fragment will allow the detection of sequence variation at this site. A similar strategy may be applied to show variants at the hybridization site of a probe. Insofar as the divergence of the target sequences or a part thereof does not affect the specificity and ubiquity of the amplification primers or probes, variant bacterial DNA is under the scope of this

invention. Variants of the selected primers or probes may also be used to amplify or hybridize to a variant DNA.

Sequencing of *tuf* sequences from a variety of bacterial, fungal and parasitical species

The nucleotide sequence of a portion of *tuf* sequences was determined for a variety of bacterial, fungal and parasitical species. The amplification primers (SEQ ID NOs: 107 and 108 in previous patent application PCT/CA97/00829), which amplify a *tuf* gene portion of approximately 890 bp, were used along with newly designed sequencing primer pairs (See Annex I for the sequencing primers for *tuf* sequences). Most primer pairs can amplify different copies of *tuf* genes (*tufA* and *tufB*). This is not surprising since it is known that for several bacterial species these two genes are nearly identical. For example, the entire *tufA* and *tufB* genes from *E. coli* differ at only 13 nucleotide positions (Neidhardt *et al.*, 1996, *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology, 2nd ed., American Society for Microbiology Press, Washington, D.C.). Similarly, some fungi are known to have two nearly identical copies of *tuf* sequences (EF-1α). These amplification primers are degenerated at several nucleotide positions and contain inosines in order to allow the amplification of a wide range of *tuf* sequences. The strategy used to select these amplification primers is similar to that illustrated in Annex I for the selection of universal primers. The *tuf* sequencing primers even sometimes amplified highly divergent copies of *tuf* genes (*tufC*) as illustrated in the case of some enterococcal species (SEQ ID NOs: 73 to 76, 614 to 618, and 621). To prove this we first had to clone PCR products before being able to sequence them. Using the cloned sequence data we designed new pair of sequencing primers specific to the divergent (*tufC*) copy of enterococci (SEQ ID NOs: 658-659 and 661) and then sequenced directly the *tufC* amplicons. The amplification primers (SEQ ID NOs: 543, 556, 557, 660, 664, 694, 696 and 697) could be used to amplify the *tuf* sequences from any bacterial species. The amplification primers (SEQ ID NOs: 558, 559, 560, 653, 654, 655, 813 and 815) could be used to amplify the *tuf* (EF-1α) genes from any fungal and parasitical species.

The *tuf* fragments to be sequenced were amplified using the following amplification protocol: One µl of cell suspension (or of purified genomic DNA 0.1-0.5 ng/µl) was transferred directly to 19 µl of a PCR reaction mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 1 µM of each of the 2 primers, 200 µM of each of the four dNTPs, 0.5 unit of *Taq* DNA polymerase (Promega Corp., Madison, WI). PCR reactions were subjected to cycling using a PTC-200 thermal cycler (MJ Research Inc., Watertown, Mass.) as follows: 3 min at 96°C followed by 30-45 cycles of 1 min at 95°C for the

denaturation step, 1 min at 30-50°C for the annealing step and 1 min at 72°C for the extension step. Subsequently, twenty microliters of the PCR-amplified mixture were resolved by electrophoresis in a 1.5% agarose gel. The gel was then visualized by staining with methylene blue (Flores *et al.*, 1992, Biotechniques, **13**:203-205). The size of the amplification products was estimated by comparison with a 100-bp molecular weight ladder. The band corresponding to the specific amplification product was excised from the agarose gel and purified using the QIAquick™ gel extraction kit (QIAGEN Inc., Chatsworth, CA). The gel-purified DNA fragment was then used directly in the sequencing protocol. Both strands of the *tuf* genes amplification product were sequenced by the dideoxynucleotide chain termination sequencing method by using an Applied Biosystems automated DNA sequencer (model 377) with their Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Corp., Applied Biosystems Division, Foster City, CA). The sequencing reactions were performed by using the same amplification primers and 10 ng/100 bp of the gel-purified amplicon per reaction. For the sequencing of long amplicons such as those of eukaryotic *tuf* (EF-1α) sequences, we designed internal sequencing primers (SEQ ID NOs: 654, 655 and 813) to be able to obtain sequence data on both strands for most of the fragment length. In order to ensure that the determined sequence did not contain errors attributable to the sequencing of PCR artifacts, we have sequenced two preparations of the gel-purified *tuf* amplification product originating from two independent PCR amplifications. For most target microbial species, the sequences determined for both amplicon preparations were identical. In case of discrepancies, a third independant PCR amplification was sequenced. Furthermore, the sequences of both strands were 100% complementary thereby confirming the high accuracy of the determined sequence. The *tuf* sequences determined using the above strategy are described in the Sequence Listing. Table 7 gives the originating microbial species and the source for each *tuf* sequence in the Sequence Listing.

The alignment of the *tuf* sequences determined by us or selected from databases reveals clearly that the length of the sequenced portion of the *tuf* genes is variable. There may be insertions or deletions of several amino acids. In addition, in several fungi introns were observed. Intron sequences are part of *tuf* sequences and could be useful in the design of species-specific primers and probes. This explains why the size of the sequenced *tuf* amplification product was variable from one species to another. Consequently, the nucleotide positions indicated on top of each of Annexes IV to XX do not correspond for sequences having insertions or deletions.

It should also be noted that the various *tuf* sequences determined by us occasionally contain degenerescences. These degenerated nucleotides correspond to sequence variations between *tufA* and *tufB* genes (or copies of EF-1α subdivision of *tuf* sequences for fungi and parasite) because the amplification primers amplify both

tuf genes. These nucleotide variations were not attributable to nucleotide misincorporations by the *Taq* DNA polymerase because the sequence of both strands was identical and also because the sequences determined with both preparations of the gel-purified *tuf* amplicons were identical.

The selection of amplification primers from *tuf* sequences

The *tuf* sequences determined by us or selected from public databases were used to select PCR primers for (i) the universal detection of bacteria, (ii) the genus-specific detection and identification of *Enterococcus* spp. and *Staphylococcus* spp. and (iii) the species-specific detection and identification of *Candida albicans*. The strategy used to select these PCR primers was based on the analysis of multiple sequence alignments of various *tuf* sequences. For more details about the selection of PCR primers from *tuf* sequences please refer to Examples and Annexes.

Sequencing of *atpD* and *recA* sequences from a variety of bacterial, fungal and parasitical species

The method use to obtain *atpD* and *recA* sequences is similar to that described above for *tuf* sequences.

The selection of amplification primers from *atpD* or *recA*

The comparison of the nucleotide sequence for the *atp*D or *rec*A genes from various bacterial fungal and parasitical species allowed the selection of PCR primers (refer to Examples 1, 2 and 6 and Annexes IV, X, XX, XXI).

DNA amplification

For DNA amplification by the widely used PCR (polymerase chain reaction) method, primer pairs were derived from proprietary DNA fragments or from database sequences. Prior to synthesis, the potential primer pairs were analyzed by using the Oligo™ 5.0 software to verify that they were good candidates for PCR amplification.

During DNA amplification by PCR, two oligonucleotide primers binding respectively to each strand of the heat-denatured target DNA from the bacterial genome are used to amplify exponentially *in vitro* the target DNA by successive thermal cycles allowing denaturation of the DNA, annealing of the primers and synthesis of new targets at each cycle (Persing *et al*, 1993, Diagnostic Molecular

Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.).

Briefly, the PCR protocols were as follow: Treated clinical specimens or standardized bacterial or fungal or parasitical suspensions (see below) or purified genomic DNA from bacteria, fungi or parasites were amplified in a 20 µl PCR reaction mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 2.5 mM $MgCl_2$, 0.4 µM of each primer, 200 µM of each of the four dNTPs and 0.5 unit of *Taq* DNA polymerase (Promega) combined with the TaqStarf™ antibody (Clontech Laboratories Inc., Palo Alto, CA). The TaqStarf™ antibody, which is a neutralizing monoclonal antibody to *Taq* DNA polymerase, was added to all PCR reactions to enhance the specificity and the sensitivity of the amplifications (Kellogg *et al.*, 1994, Biotechniques 16:1134-1137). The treatment of the clinical specimens varies with the type of specimen tested, since the composition and the sensitivity level required are different for each specimen type. It consists in a rapid protocol to lyse the bacterial cells and eliminate the PCR inhibitory effects. For amplification from bacterial or fungal cultures or from purified genomic DNA, the samples were added directly to the PCR amplification mixture without any pre-treatment step. An internal control was derived from sequences not found in the target microorganisms or in the human genome. The internal control was integrated into all amplification reactions to verify the efficiency of the PCR assays and to ensure that significant PCR inhibition was absent. Alternatively, an internal control derived from rRNA was also useful to monitor the efficiency of microbial lysis protocols.

PCR reactions were then subjected to thermal cycling (3 min at 95°C followed by 30 cycles of 1 second at 95°C for the denaturation step and 30 second at 50-65°C for the annealing-extension step) using a PTC-200 thermal cycler (MJ Research Inc.). The number of cycles performed for the PCR assays varies according to the sensitivity level required. For example, the sensitivity level required for microbial detection directly from clinical specimens is higher for blood specimens than for urine specimens because the concentration of microorganisms associated with a septicemia can be much lower than that associated with a urinary tract infection. Consequently, more sensitive PCR assays having more thermal cycles are required for direct detection from blood specimens. Similarly, PCR assays performed directly from bacterial or fungal or parasitical cultures may be less sensitive than PCR assays performed directly from clinical specimens because the number of target organisms is normally much lower in clinical specimens than in microbial cultures.

The person skilled in the art of DNA amplification knows the existence of other rapid amplification procedures such as ligase chain reaction (LCR), transcription-mediated amplification (TMA), self-sustained sequence replication (3SR), nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA), branched DNA (bDNA), cycling probe technology (CPT), solid phase

amplification (SPA) and nuclease dependant signal amplification (NDSA) (Lee*et al.*, 1997, Nucleic Acid Amplification Technologies: Application to Disease Diagnosis, Eaton Publishing, Boston, MA; Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). The scope of this invention is not limited to the use of amplification by PCR, but rather includes the use of any rapid nucleic acid amplification method or any other procedure which may be used to increase rapidity and sensitivity of the nucleic acid-based tests. Any oligonucleotide suitable for the amplification of nucleic acids by approaches other than PCR or for DNA hybridization and derived from the species-specific, genus-specific and universal DNA fragments as well as from selected antibiotic resistance or toxin gene sequences included in this document are also under the scope of this invention.

Detection of amplification products

Classically, detection of amplification is performed by standard ethidium bromide-stained agarose gel electrophoresis. It is clear that other methods for the detection of specific amplification products, which may be faster and more practical for routine diagnosis, may be used. Such methods may be based on the detection of fluorescence after or during amplification. One simple method for monitoring amplified DNA is to measure its rate of formation by measuring the increase in fluorescence of intercalating agents such as ethidium bromide or SYBR® Green I. If more specific detection is required, fluorescence based technologies can monitor the appearance of a specific product during the reaction. The use of dual-labeled fluorogenic probes such as in the TaqMan™ system which utilizes the 5'-3' exonuclease activity of the *Taq* polymerase is a good example (Livak K.J.*et al.* 1995, PCR Methods Appl. 4:357-362). TaqMan™ can be performed during amplification and this "real-time" detection can be done in a single closed tube hence eliminating post-PCR sample handling and consequently preventing the risk of amplicon carryover (TaqMan™ system from Perkin Elmer or Amplisensor™ from Biotronics). Several other fluorescence-based detection methods can be performed in real-time. Fluorescence resonance energy transfer (FRET) is the principle behind the use of adjacent hybridization probes and molecular beacons. Adjacent hybridization probes are designed to be internal to the amplification primers. The 3' end of one probe is labelled with a donor fluorophore while the 5' end of an adjacent probe is labelled with an acceptor fluorophore. When the two probes are specifically hybridized in closed proximity (spaced by 1 to 5 nucleotides) the donor fluorophore which has been excited by an external light source emits light that is absorbed by a second, acceptor that emit more fluorescence and yield FRET signal. Molecular beacons possess a stem-and-loop structure where the loop is the probe and at the end of the stem a

fluorescent moiety is at one end while a quenching moiety is at the other end. The beacons undergo a fluorogenic conformational change when they hybridize to their targets hence separating the fluorochrome from its quencher. The FRET principle is also used in an air thermal cycle with a built-in fluorometer (Wittwer, C.T. *et al.* 1997. BioTechniques. 22:130-138). The amplification and detection are extremely rapid as reactions are performed in capillaries and it takes 18 min to complete 45 cycles. Those techniques are suitable, especially in the case where few pathogens are searched for. Boerhinger-Roche Inc., sells the LightCycler™, an apparatus capable of rapid cycle PCR combined with fluorescent SYBR® Green I or FRET detection. We recently demonstrated in our laboratory, real-time detection of 10 CFU in less than 40 minutes using adjacent hybridization probes on the LightCycler™.Methods based on the detection of fluorescence are particularly promising for utilization in routine diagnosis as they are very rapid, quantitative and can be automated.

Microbial pathogens detection and identification may also be performed by solid support or liquid hybridization using species-specific internal DNA probes hybridizing to an amplification product. Such probes may be generated from any sequences from our repertory and designed to specifically hybridize to DNA amplification products which are objects of the present invention. Alternatively, the internal probes for species or genus or family or group detection and identification may be derived from the amplicons produced by a universal, family, group or genus amplification assay. The oligonucleotide probes may be labeled with biotin or with digoxigenin or with any other reporter molecules (for more details see below the section on hybrid capture). Hybrization on solid support is amendable to miniaturization.

At present the oligonucleotide nucleic acid microarray technology is appealing. Currently, available low to medium density arrays (Heller *et al.*, An integrated microelectronics hybridization system for genomic research and diagnostic applications. *In*: Harrison, D.J., and van den Berg, A., 1998, Micro total analysis systems '98, Kluwer Academic Publisher, Dordrecht.) could specifically capture fluorescent-labelled amplicons. Detection methods for hybridization are not limited to fluorescence, potentiometry, colorimetry and plasmon resonance are some examples of alternative detection methods. In addition to detection by hybridization, nucleic acid microarrays could be use to perform rapid sequencing by hybrization. Mass spectrometry could also be applicable for rapid identification of the amplicon or even for sequencing of the amplification products (Chiu and Cantor, 1999, Clinical Chemistry **45**:1578; Berkenkamp *et al.*, 1998, Science **281**:260).

We also keep in mind the major challenge of molecular diagnostics tools,*i.e.* : integration of the major steps including sample preparation, genetic amplification, detection, data analysis and presentation (Anderson *et al.*, Advances in integrated genetic analysis. *In*: Harrison, D.J., and van den Berg, A., 1998, Micro total analysis systems '98, Kluwer Academic Publisher, Dordrecht.).

To assure PCR efficiency, glycerol, dimethyl sulfoxide (DMSO) or other related solvents can be used to increase the sensitivity of the PCR and to overcome problems associated with the amplification of a target DNA having a high GC content or forming strong secondary structures (Dieffenbach and Dveksler, 1995, PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, New York). The concentration ranges for glycerol and DMSO are 5-15% (v/v) and 3-10% (v/v), respectively. For the PCR reaction mixture, the concentration ranges for the amplification primers and $MgCl_2$ are 0.1-1.5 µM and 1.0-10.0 mM, respectively. Modifications of the standard PCR protocol using external and nested primers (i.e. nested PCR) or using more than one primer pair (i.e. multiplex PCR) may also be used (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). For more details about the PCR protocols and amplicon detection methods, see Examples.

Hybrid capture and chemiluminescence detection of amplification products

Hybridization and detection of amplicons by chemiluminescence were adapted from Nikiforov *et al.* (1994, PCR Methods and Applications 3:285-291 and 1995, Anal. Biochem. 227:201-209) and from the DIG™ system protocol of Boehringer Mannheim. Briefly, 50 µl of a 25 picomoles solution of capture probe diluted in EDC {1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride} are immobilized in each well of 96 wells plates (Microlite™ 2, Dynex) by incubation overnight at room temperature. The next day, the plates are incubated with a solution of 1% BSA diluted into TNTw (10 mM Tris-HCl, pH 7.5; 150 mM NaCl; 0.05% Tween™ 20) for 1 hour at 37°C. The plates are then washed on a Wellwash Ascent™ (Labsystems) with TNTw followed by Washing Buffer (100 mM maleic acid; 150 mM NaCl; pH7.5; 0.3% Tween™ 20).

The amplicons were labelled with DIG-11-dUTP during PCR using the PCR DIG Labelling Mix from Boehringer Mannheim according to the manufacturer's instructions. Hybridization of the amplicons to the capture probes is performed in triplicate at stringent temperature (generally, probes are designed to allow hybrization at 55°C, the stringent temperature) for 30 minutes in 1.5 M NaCl; 10 mM EDTA. It is followed by two washes in 2 X SSC; 0.1% SDS, then by four washes in 0.1X SSC; 0.1% SDS at the stringent temperature (55°C). Detection with 1,2 dioxetane chemiluminescent alkaline phosphatase substrates like CSPD® (Tropix inc.) is performed according to the manufacturer's instructions but with shorter incubations times and a different antibody concentration. The plates are agitated at each steps, the blocking incubation is performed for only 5 minutes, the anti-DIG-AP1 is used at a 1:1000 dilution, the incubation with antibody last 15 minutes, the plates are washed twice for only 5 minutes. Finally, after a 2 minutes incubation into the detection

buffer, the plates are incubated 5 minutes with CSPD® at room temperature followed by a 10 minutes incubation at 37°C without agitation. Luminous signal detection is performed on a Dynex Microtiter Plate Luminometer using RLU (Relative Light Units).

Specificity and ubiquity tests for oligonucleotide primers and probes

The specificity of oligonucleotide primers and probes was tested by amplification of DNA or by hybridization with bacterial or fungal or parasitical species selected from a panel comprising closely related species and species sharing the same anatomo-pathological site (see Annexes and Examples). All of the bacterial, fungal and parasitical species tested were likely to be pathogens associated with infections or potential contaminants which can be isolated from clinical specimens. Each target DNA could be released from bacterial cells using standard chemical and/or physical treatments to lyse the cells (Sambrook *et al.*, 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY) or alternatively, genomic DNA purified with the GNOME™ DNA kit (Bio101, Vista, CA) was used. Subsequently, the DNA was subjected to amplification with the primer pairs.

Oligonucleotides primers found to amplify specifically the target species, genus, family or group were subsequently tested for their ubiquity by amplification (i.e. ubiquitous primers amplified most or all isolates of the target species or genus or family or group). The specificity and ubiquity of the PCR assays using the selected amplification primer pairs were tested either directly from cultures of microbial species or from purified microbial genomic DNA.

Probes were tested in hybrid capture assays as described above. An oligonucleotide probe was considered specific only when it hybridized solely to DNA from the species or genus or family or group from which it was selected. Oligonucleotide probes found to be specific were subsequently tested for their ubiquity (i.e. ubiquitous probes recognized most or all isolates of the target species or genus or family or group) by hybridization to microbial DNAs from different clinical isolates of the species or genus or family or group of interest including ATCC reference strains. Similarly, oligonucleotide primers and probes could be derived from antibiotic resistance or toxin genes which are objects of the present invention.

Reference strains

The reference strains used to built proprietary *tuf*, *atpD* and *recA* sequence data repertory, as well as to test the amplification and hybridization assays were obtained from (i) the American Type Culture Collection (ATCC), (ii) the Laboratoire de santé

publique du Québec (LSPQ), (iii) the Centers for Disease Control and Prevention (CDC), (iv) the National Culture Type Collection (NCTC) and (v) several other reference laboratories throughout the world. The identity of our reference strains was confirmed by phenotypic testing and reconfirmed by analysis of *tuf*, *atpD* and *recA* sequences (see example 13).

Antibiotic resistance genes

Antimicrobial resistance complicates treatment and often leads to therapeutic failures. Furthermore, overuse of antibiotics inevitably leads to the emergence of bacterial resistance. Our goal is to provide clinicians, in approximately one hour, the needed information to prescribe optimal treatments. Besides the rapid identification of negative clinical specimens with DNA-based tests for universal bacterial detection and the identification of the presence of a specific pathogen in the positive specimens with species- and/or genus- and/or family- and/or group-specific DNA-based tests, clinicians also need timely information about the ability of the bacterial pathogen to resist antibiotic treatments. We feel that the most efficient strategy to evaluate rapidly bacterial resistance to antimicrobials is to detect directly from the clinical specimens the most common and clinically important antibiotic resistance genes (i.e. DNA-based tests for the detection of antibiotic resistance genes). Since the sequence from the most important and common bacterial antibiotic resistance genes are available from public databases, our strategy is to use the sequence from a portion or from the entire resistance gene to design specific oligonucleotide primers or probes which will be used as a basis for the development of rapid DNA-based tests. The list of each of the bacterial antibiotic resistance genes selected on the basis of their clinical relevance (i.e. high incidence and importance) is given in the Table 5. Our approach is unique because the antibiotic resistance genes detection and the bacterial detection and identification can be performed simultaneously in multiplex assays under uniform PCR amplification conditions .

Toxin genes

Toxin identification is often very important to prescribe optimal treatments. Besides the rapid identification of negative clinical specimens with DNA-based tests for universal bacterial detection and the identification of the presence of a specific pathogen in the positive specimens with species- and/or genus- and/or family- and/or group-specific DNA-based tests, clinicians sometimes need timely information about the ability of certain bacterial pathogen to produce toxins. Since the sequence from the most important and common bacterial toxin genes are available from public databases, our strategy is to use the sequence from a portion or from the entire toxin

gene to design specific oligonucleotide primers or probes which will be used as a basis for the development of rapid DNA-based tests. The list of each of the bacterial toxin genes selected on the basis of their clinical relevance (i.e. high incidence and importance) is given in the Table 6. Our approach is unique because the toxin genes detection and the bacterial detection and identification can be performed simultaneously in multiplex assays under uniform PCR amplification conditions .

Universal bacterial detection

In the routine microbiology laboratory, a high percentage of clinical specimens sent for bacterial identification are negative by culture. Testing clinical samples with universal amplification primers or universal probes to detect the presence of bacteria prior to specific identification and screen out the numerous negative specimens is thus useful as it saves costs and may rapidly orient the clinical management of the patients. Several amplification primers and probes were therefore synthesized from highly conserved portions of bacterial sequences from the *tuf* and *atpD* sequences. The universal primers selection was based on a multiple sequence alignment constructed with sequences from our repertory.

All computer analysis of amino acid and nucleotide sequences were performed by using the GCG programs. Subsequently, optimal PCR primers for the universal amplification of bacteria were selected with the help of the Oligo™ program. The selected primers are degenerated at several nucleotide positions and contain several inosines in order to allow the amplification of all clinically relevant bacterial species (Annex I). Inosine is a nucleotide analog able to specifically bind to any of the four nucleotides A, C, G or T. Degenerated oligonucleotides consist of an oligonucleotide mix having two or more of the four nucleotides A, C, G or T at the site of mismatches. The inclusion of inosine and/or of degenerescences in the amplification primers allow mismatch tolerance thereby permitting the amplification of a wider array of target nucleotide sequences (Dieffenbach and Dveksler, 1995 PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, NY).

The amplification conditions with the universal primers are very similar to those used for the species- and genus-specific amplification assays except that the annealing temperature is slightly lower. The original universal PCR assay described in our co-pending PCT (PCT/CA97/00829)(SEQ ID NO. 23-24 of the latter application) was specific and nearly ubiquitous for the detection of bacteria. The specificity for bacteria was verified by amplifying genomic DNA isolated from the 12 fungal species as well as genomic DNA from *Leishmania donovani*, *Saccharomyces cerevisiae* and human lymphocytes. None of the above eukaryotic DNA preparations could be amplified by the universal assay, thereby suggesting that

this test is specific for bacteria. The ubiquity of the universal assay was verified by amplifying genomic DNAs from 116 reference strains which represent 95 of the most clinically relevant bacterial species. These species have been selected from the bacterial species listed in Table 4. We found that at least 104 of these species could be amplified. However, the assay could be improved since bacterial species which could not be amplified with the original *tuf* sequences-based assay included species belonging to the following genera: *Corynebacterium* (11 species) and *Stenotrophomonas* (1 species). Sequencing of the *tuf* genes from these bacterial species and other has been performed in the scope of the present invention in order to improve the universal assay. This sequencing data has been used to select new universal primers which may be more ubiquitous. Also, we improved our primer and probes design strategy by taking into consideration the phylogeny observed in analysing our repertory of *tuf*, *atpD* and *recA* sequences. Data from each of the 3 main subrepertory (*tuf*, *atpD* and *recA*) was subjected to a basic phylogenic analysis using the Pileup command from version 10 of the GCG package (Genetics Computer Group, inc.). This analysis indicated the main branches or phyla reflecting the relationships between sequences. Instead of trying to design primers or probes able to hybridize to all phyla, we designed primers or probes able to hybridize to the main phyla while trying to use the largest phylum possible. This strategy should allow less degenerated primers hence improving sensitivity and by combining primers in a mutiplex assay, improve ubiquity. Universal primers SEQ ID NO. 643-645 based on *tuf* sequences have been designed to amplify most pathogenic bacteria except *Actinomyceteae*, *Clostridiaceae* and the *Cytophaga*, *Flexibacter* and *Bacteroides* phylum (pathogenic bacteria of this phylum include mostly *Bacteroides*, *Porphyromonas* and *Prevotella* species). Primers to fill these gaps have been designed for *Actinomyceteae* (SEQ ID NO. 646-648), *Clostridiaceae* (SEQ ID NO. 796-797, 808-811), and the *Cytophaga*, *Flexibacter* and *Bacteroides* phylum (SEQ ID NO. 649-651). These primers sets could be used alone or in conjuction to render the universal assay more ubiquitous. These primers are in the process of being tested.

Universal primers derived from *atpD* sequences include SEQ ID NO. 562-565. Combination of these primers does not amplified human DNA but should amplify almost all pathogenic bacterial species except proteobacteria belonging to the epsilon subdivision (*Campylobacter* and *Helicobacter*), the bacteria from the *Cytophaga*, *Flexibacter* and *Bacteroides* group and some actinomycetes and corynebacteria. By analysing *atpD* sequences from the latter species, primers and probes to specifically fill these gaps could be designed and used in conjuction with primers SEQ ID NO. 562-565. These primers are in the process of being tested.

In addition, universality of the assay could be expanded by mixing *atpD* sequences-derived primers with *tuf* sequences-derived primers. Ultimately, even *recA* sequences-derived primers could be added to fill some gaps in the universal assay.

It is important to note that the 95 bacterial species selected to test the ubiquity of the universal assay include all of the most clinically relevant bacterial species associated with a variety of human infections acquired in the community or in hospitals (nosocomial infections). The most clinically important bacterial and fungal pathogens are listed in Tables 1 and 2.

Amino acid sequences derived from *atpD*, *tuf* and *recA* sequences

The amino acid sequences translated from the repertory of *atpD*, *tuf* and *recA* sequences are also an object of the present invention. The amino acid sequence data will be particularly useful for homology modeling of three-dimensional (3D) structure of the elongation factor-Tu, Atpase subunit beta and RecA recombinase. For all three proteins, at least one structure model as been published using X-ray diffraction data from crystals. Based on those structural informations it is possible to use computer sofwares to build model 3D structures for any other proteins having peptide sequence homologies with the known structure (Greer, 1991, Methods in Enzymology, **202**:239-252, Taylor, 1994, Sali, 1995, Curr. Opin. Biotechnol.**6**:437-451, Sanchez and Sali, 1997, Curr. Opin. Struct. Biol. 7:206-214, Fischer and Eisenberg, 1999, Curr. Opin. Struct. Biol. **9**:208-211, Guex *et al.*, 1999, Trends Biochem. Sci. **24**: 364-367). Model structures of target proteins are used for the design or to predict the behavior of ligands and inhibitors such as antibiotics. Since EF-Tu is already known as an antibiotic target (see above) and since the beta subunit of ATPase and RecA recombinase are essential to the survival of the microbial cells in natural conditions of infection, all three proteins could be considered antibiotic targets. Sequence data, especially the new data generated by us could be very useful to assist the creation of new antibiotic molecules with desired spectrum of activity. In addition, model structures could be used to improved protein function for commercial purposes such as improving antibiotic production by microbial strains or increasing biomass.

BRIEF SUMMARY OF THE INVENTION

Three highly conserved genes, encoding translation elongation factor Tu, the catalytic subunit of proton-translocating ATPase and the RecA recombinase, are used to generate species-specific, genus-specific, group-specific and universal nucleic acid probes and amplification primers to rapidly detect and identify bacterial, fungal and parasitical pathogens from clinical specimens for diagnosis. The concomittant detection of associated antibiotic resistance and toxin genes are also under the scope of the present invention.

DESCRIPTION OF THE DRAWINGS

Figures 1 and 2 illustrate the principal subdivisions of the *atpD* and *tuf* sequences repertories, respectively. For the design of primers and probes, depending on the needs, one may want to use the complete data set illustrated on the top of the pyramid or use only a subset illustrated by the different branching points. Smaller subdivisions, representing groups, families, genus and species, could even be made to extend the bottom of the pyramid. Because the *atpD* and *tuf* sequences are highly conserved and evolved with each species, the design of primers and probes does not need to include all the sequences within the database or its subdivisions. As illustrated, in Annexes IV to XX, depending on the use, sequences from a limited number of species can be carefully selected to represent: i) only the main phylogenetic branches from which the intended probes and primers need to be differentiating, and ii) only the species for which they need to be matching. However, for ubiquity purposes, and especially for primers and probes identifying large groups of species (genus, family, group or universal, or sequencing primers), the more data is included into the sequence analysis, the better the probes and primers will be suitable for each particular intented use. Similarly, for specificity purposes, a larger data set (or repertory) ensures optimal primers and probes design by reducing the chance of employing nonspecific oligonucleotides.

EXAMPLES AND ANNEXES

The following examples and annexes are intended to be illustrative of the various methods and compounds of the invention, rather than limiting the scope thereof.

The various annexes show the strategies used for the selection of amplification primers from *tuf* sequences or from the *atpD* sequences or from the *recA* sequences: (i) Annex I illustrates the amplification primers used for *tuf* sequences. (ii) Annex II illustrates the amplification primers used for *atpD* sequences. (iii) Annex III shows the probes for hybridization with *tuf* sequences. (iv) Annex IV illustrates the strategy used for the selection of the amplification primers specific for *atpD* sequences of the F-type. (v) Annex V illustrates the strategy used for the selection of the amplification primers specific for *atpD* sequences of the V-type. (vi) Annex VI illustrates the strategy used for the selection of the amplification primers specific for the *tuf* sequences of organelle lineage (M, the letter M is used to indicate that in most case, the organelle is the mitochondria). (vii) Annexes VII illustrates the strategy used for the selection of the amplification primers specific for the *tuf* sequences of eukaryotes (*ef-1*). (viii) Annex VIII illustrates the strategy for the selection of *Streptococcus agalactiae*-specific amplification primers from *tuf* sequences. (ix) Annex IX illustrates the strategy for the selection of *Streptococcus agalactiae*-specific hybridization probes from *tuf* sequences. (x) Annex X illustrates the strategy for the selection of *Streptococcus agalactiae*-specific amplification primers from *atp*D sequences. (xi) Annex XI illustrates the Strategy for the selection from *tuf* sequences of *Candida albicans/dubliniensis*-specific amplification primers, *Candida albicans*-specific hybridization probe and *Candida dubliniensis*-specific hybridization probe. (xii) Annex XII illustrates the strategy for the selection of *Staphylococcus*-specific amplification primers from *tuf* sequences. (xiii) Annex XIII illustrates the Strategy for the selection of the *Staphylococcus* genus-specific hybridization probe from *tuf* sequences. (xiv) Annex XIV illustrates the strategy for the selection of *Staphylococcus saprophyticus*-specific and of *Staphylococcus haemolyticus*-specific hybridization probes from *tuf* sequences. (xv) Annex XV illustrates the strategy for the selection of *Staphylococcus aureus*-specific and of *Staphylococcus epidermidis*-specific hybridization probes from *tuf* sequences. (xvi) Annex XVI illustrates the strategy for the selection of the *Staphylococcus hominis*-specific hybridization probe from *tuf* sequences. (xvii) Annex XVII illustrates the strategy for the selection from *tuf* sequences of the amplification primers specific for the genus *Enterococcus*. (xviii) Annex XVIII illustrates the strategy for the selection of the *Enterococcus faecalis*-specific hybridization probe, of the *Enterococcus faecium*-specific hybridization probe and of the *Enterococcus casseliflavus-flavescens-gallinarum*

group-specific hybridization probe from *tuf* sequences. (xix) Annex XIX illustrates the strategy for the selection of primers from *tuf* sequences for the identification of platelets contaminants. (xx) Annex XX illustrates the strategy for the selection of the universal amplification primers from *atp*D sequences. (xxi) Annex XXI illustrates the amplification primers used for DNA amplification from *rec*A sequences.

As shown in these annexes, the selected amplification primers may contain inosines and/or degenerescences. Inosine is a nucleotide analog able to specifically bind to any of the four nucleotides A, C, G or T. Alternatively, degenerated oligonucleotides which consist of an oligonucleotide mix having two or more of the four nucleotides A, C, G or T at the site of mismatches were used. The inclusion of inosine and/or of degenerescences in the amplification primers allow mismatch tolerance thereby permitting the amplification of a wider array of target nucleotide sequences (Dieffenbach and Dveksler, 1995 PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, New York).

EXAMPLES

EXAMPLE 1:

Sequencing of bacterial *atpD* (F-type) gene fragments. As shown in Annex IV, the comparison of publicly available *atpD* (F-type) sequences from a variety of bacterial species revealed conserved regions allowing the design of PCR primers able to amplify *atpD* sequences from a wide range of bacterial species. Using primers pairs SEQ ID NO. 566 and 567, 566 and 814, 568 and 567, 570 and 567, 572 and 567, 569 and 567, 571 and 567, and 700 and 567, it was possible to amplify and sequence *atpD* sequences SEQ ID NO. 242-270, 272-398, 673-674, 737-767, and 866-867.

EXAMPLE 2:

Sequencing of eukaryotic *atpD* (F-type) gene fragments. The comparison of publicly available *atpD* (F-type) sequences from a variety of fungal and parasitical species revealed conserved regions allowing the design of PCR primers able to amplify *atpD* sequences from a wide range of fungal and parasitical species. Using primers pairs SEQ ID NO. 568 and 573, 574 and 573, and 574 and 708, it was possible to amplify and sequence *atpD* sequences SEQ ID NO. 458-497, 530-538, 663, 667, 676, 678-680, 768-778, 856-862, and 889-896.

EXAMPLE 3:

Sequencing of eukaryotic *tuf* (ef-1) gene fragments. As shown in Annex VII, the comparison of publicly available *tuf* (ef-1) sequences from a variety of fungal and parasitical species revealed conserved regions allowing the design of PCR primers able to amplify *tuf* sequences from a wide range of fungal and parasitical species. Using primers pairs SEQ ID NO. 558 and 559, 813 and 559, 558 and 815, 560 and 559, 653 and 559, 558 and 655, and 654 and 559, it was possible to amplify and sequence *tuf* sequences SEQ ID NO. 399-457, 509-529, 622-624, 677, 779-790, 840-842, 865, and 897-903.

EXAMPLE 4:

Sequencing of eukaryotic *tuf* (organelle origin, M) gene fragments. As shown in Annex VI, the comparison of publicly available *tuf* (organelle origin, M) sequences from a variety of fungal and parasitical organelles revealed conserved regions allowing the design of PCR primers able to amplify *tuf* sequences of several organelles belonging to a wide range fungal and parasitical species. Using primers pairs SEQ ID NO. 664 and 652, 664 and 561, 911 and 914, 912 and 914, 913 and 915, 916 and 561, and 664 and 917, it was possible to amplify and sequence *tuf* sequences SEQ ID NO. 498-508, 791-792, 843-855, and 904-910.

EXAMPLE 5:

Specific identification of *Streptococcus agalactiae* using *tuf* sequences. As shown in annex VIII, the comparison of *tuf* sequences from a variety of bacterial species allowed the selection of PCR primers specific for *S. agalactiae*. The strategy used to design the PCR primers was based on the analysis of a multiple sequence alignment of various *tuf* sequences. The multiple sequence alignment include the *tuf* sequences of four bacterial strains from the target species as well as *tuf* sequences from other species and bacterial genera especially, representative of closely related species. A careful analysis of this alignment allowed the selection of oligonucleotide sequences which are conserved within the target species but which discriminate sequences from other species and genera, especially from the closely related species, thereby permitting the species-specific and ubiquitous detection and identification of the target bacterial species.

The chosen primer pair, oligos TSag340 (SEQ ID NO. 549) and TSag552 (SEQ ID NO. 550), gives an amplification product of 252 bp. Standard PCR was carried out using 0.4 µM of each primers, 2.5 mM $MgCl_2$, BSA 0.05 mM, 1X Taq Buffer (Promega), dNTP 0.2 mM (Pharmacia), 1 µl Taq DNA polymerase (Promega) 0.025 U/µl combined with TaqStart 5 ng/µl (Clontech Laboratories Inc., Palo Alto), 1 µl of genomic DNA sample in a final volume of 20 µl using a PTC-200 thermocycler (MJ Research Inc.). The optimal cycling conditions for maximum sensitivity and

specificity were 3 minutes at 95°C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95°C and 30 seconds at 62°C, followed by terminal extension at 72°C for 2 minutes. Amplification was monitored on agarose gel electrophoresis by staining the DNA with ethidium bromide.

Specificity of the assay was tested by adding into the PCR reactions, 0.1 ng of genomic DNA from each of the bacterial species listed in Table 8. Strong amplification was observed only for the 5 *S. agalactiae* strains listed. Of the other bacterial species, including 32 species representative of the vaginal flora and 27 other streptococcal species, only *S. acidominimus* yielded amplification. The signal for 0,1 ng of *S. acidominimus* genomic DNA was weak and the detection limit for this species was 10 pg (corresponding to more than 4000 genome copies) while the detection limit for *S. agalactiae* was 2.5 fg (corresponding to one genome copy) of genomic DNA.

To increase the specificity of the assay, internal probes were designed for FRET (Fluorescence Resonance Energy Transfer) detection using the LightCycler (Idaho Technology). As illustrated in annex IX, a multiple sequence alignment of streptococcal *tuf* sequence fragments corresponding the 252 bp region amplified by primers TSag340 (SEQ ID NO. 549) and TSag552 (SEQ ID NO. 550), was used for the design of internal probes TSagHF436 (SEQ ID NO. 582) and TSagHF465 (SEQ ID NO. 583). The region of the amplicon selected for internal probes contained sequences unique and specific to *S. agalactiae*. TSagHF465 (SEQ ID NO. 583), the more specific probe is labelled with fluorescein in 3' while TSagHF436 (SEQ ID NO. 582), the less discriminant probe is labelled with CY5 in 5' and blocked in 3' with a phosphate group. However, since the FRET signal is only emitted if both probes are adjacently hybridized on the same target amplicon, detection is highly specific.

Real-time detection of PCR products using the LightCycler™ was carried out using 0.4 µM of each primers (SEQ ID NO. 549-550), 0,2 µM of each probes (SEQ ID NO. 582-583), 2.5 mM $MgCl_2$, BSA 450 µg/ml, 1X PC2 Buffer (AB Peptides, St-Louis, MO), dNTP 0.2 mM (Pharmacia), KlenTaq1™ DNA polymerase 0.5 U (AB Peptides) 0.025 U/µl combined with TaqStart (Clontech Laboratories Inc., Palo Alto), 0.7 µl of genomic DNA sample in a final volume of 7 µl using a LightCycler thermocycler (Idaho Technology). The optimal cycling conditions for maximum sensitivity and specificity were 3 minutes at 94°C for initial denaturation, then forty cycles of three steps consisting of 0 second (this setting meaning the LightCycler will reach the target temperature and stay at it for its minimal amount of time) at 94°C, 10 seconds at 64°C, 20 seconds at 72°C. Amplification was monitored during each

annealing steps using the fluorescence ratio. The streptococcal species having close sequence homologies with the *tuf* sequence of *S. agalactiae* (*S. acidominimus, S. anginosus, S. bovis, S. dysgalactiae, S. equi, S. ferus, S. gordonii, S. intermedius, S. parasanguis, S. parauberis, S. salivarius, S. sanguis, S. suis*, and of course *S. agalactiae*) were tested in the LightCycler with 0.07 ng of genomic DNA per reaction. This time, only *S. agalactiae* yielded an amplification signal, hence demonstrating that the assay is species-specific. With the LightCycler assay using the internal FRET probes, the detection limit for *S. agalactiae* was 12.5 fg (corresponding to five genome copies) of genomic DNA.

EXAMPLE 6:

Specific identification of *Streptococcus agalactiae* using *atpD* sequences. As shown in Annex XIV, the comparison of *atpD* sequences from a variety of bacterial species allowed the selection of PCR primers specific for *S. agalactiae*. The primer design strategy is similar the strategy described in the preceeding example except that *atpD* sequences were used in the alignment (see Annex X).

Four primers were selected, ASag42 (SEQ ID NO. 627), ASag52 (SEQ ID NO. 628), ASag206 (SEQ ID NO. 625) and ASag371 (SEQ ID NO. 626). The following combinations of these four primers give four amplicons; SEQ ID NO. 627 + SEQ ID NO. 625 = 190 bp, SEQ ID NO. 628 + SEQ ID NO. 625 = 180 bp, SEQ ID NO. 627 + SEQ ID NO. 626 = 355 bp, and SEQ ID NO. 628 + SEQ ID NO. 626 = 345 bp.

Standard PCR was carried out on PTC-200 thermocyclers (MJ Research Inc) using 0.4 µM of each primers pairs, 2.5 mM $MgCl_2$, BSA 0.05 mM, 1X Taq Buffer (Promega), dNTP 0.2 mM (Pharmacia), 1 µl Taq DNA polymerase (Promega) 0.025 U/µl combined with TaqStart 5 ng/µl (Clontech Laboratories Inc., Palo Alto), 1 µl of genomic DNA sample in a final volume of 20 µl using a PTC-200 thermocycler (MJ Research Inc.). The optimal cycling conditions for maximum sensitivity and specificity were adjusted for each primer pairs. Three minutes at 95°C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95°C and 30 seconds at the optimal temperature specified below, followed by terminal extension at 72°C for 2 minutes. Amplification was monitored on agarose gel electrophoresis by staining the DNA with ethidium bromide. Since *atpD* sequences are relatively more specific than *tuf* sequences, only the more closely related species namely, the steptococcal species listed in table 9, were tested.

All four primer pairs only amplified the six *S. agalactiae* strains. With an annealing temperature of 63°C, the primer pair SEQ ID NO. 627 + SEQ ID NO. 625 had a

sensitivity of 1-5 fg (equivalent to 1-2 genome copies). At 55°C, the primer pair SEQ ID NO. 628 + SEQ ID NO. 625 had a sensitivity of 2.5 fg (equivalent to 1 genome copy). At 60°C, the primer pair SEQ ID NO. 627 + SEQ ID NO. 626 had a sensitivity of 10 fg (equivalent to 4 genome copies). At 58°C, the primer pair SEQ ID NO. 628 + SEQ ID NO. 626 had a sensitivity of 2.5-5 fg (equivalent to 1-2 genome copies). This proves that all four primer pairs can detect *S. agalactiae* with high specificity and sensitivity. Together with example 5, this example demonstrate that both *tuf* and *atpD* sequences are suitable targets for the identification of microorganisms at the species level.

EXAMPLE 7:

Development of a PCR Assay for Detection and Identification of Staphylococci at Genus and Species Levels.

Material and Methods

Bacterial strains. The specificity of the PCR assay was verified by using a panel of ATCC (America Type Culture Collection) and DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH ; German Collection of Microorganisms and Cell Cultures) (reference strains consisting of 33 gram-negative and 47 gram-positive bacterial species (Table 11). An additional 295 clinical isolates of 11 different species of staphylococci from the microbiology laboratory of the Centre Hospitalier Universitaire de Québec, Pavillon Centre Hospitalier de l'Université Laval (CHUL) (Ste-Foy, Québec, Canada) were also tested to further validate the *Staphylococcus*-specific PCR assay. These strains were all identified by using (i) conventional methods or (ii) the automated MicroScan Autoscan-4 system equipped with the Positive BP Combo Panel Type 6 (Dade Diagnostics, Mississauga, Ontario, Canada). Bacterial strains were grown from frozen stocks kept at –80°C in brain heart infusion (BHI) broth containing 10% glycerol and cultured on sheep blood agar or in BHI broth (Quelab Laboratories Inc, Montréal, Québec, Canada).

PCR primers and internal probes. Based on multiple sequence alignments, regions of the *tuf* gene unique to staphylococci were identified. *Staphylococcus* genus-specific PCR primers TStaG422 (SEQ ID NO. 553) and TStaG765 (SEQ ID NO. 575) were derived from these regions (Annex XII). These PCR primers are displaced by two nucleotide positions compared to original *Staphylococcus* genus-specific PCR primers described in previous patent application WO 98/20157 (SEQ ID NO. 17 and 20 in the said patent application). These modifications were done to ensure specificity and ubiquity of the primer pair, in the light of new *tuf* sequence data revealed in the present patent application for several additional streptococcal species and strains.

Similarly, sequence alignments analysis were performed to design genus and species-specific internal probes. Two internal probes for *Staphylococcus*-genus (SEQ ID NO. 605-606), five for *S. aureus* (SEQ ID NO. 584-588), five for *S. epidermidis* (SEQ ID NO. 589-593), two for *S. haemolyticus* (SEQ ID NO. 594-595), three for *S. hominis* (SEQ ID NO. 596-598), four for *S. saprophyticus* (SEQ ID NO. 599-601 and 695) were designed. The range of mismatches between *Staphylococcus*-specific 371-bp amplicon and each of the 20-mer species-specific internal probes was from 1 to 5, in the middle of the probe when possible. No mismatches were present in the two *Staphylococcus*-genus probes for the 11 species analyzed; *S. aureus, S. auricularis, S. capitis, S. cohnii, S. epidermidis, S. haemolyticus, S. hominis, S. lugdunensis, S. saprophyticus, S. simulans* and *S. warneri*. In order to verify the intra-specific sequence conservation of the nucleotide sequence, sequences were obtained for the 371-bp amplicon from five unrelated ATCC and clinical strains for each of the species *S. aureus, S. epidermidis, S. haemolyticus, S. hominis* and *S. saprophyticus*. The Oligo™ (version 5.0) primer analysis software (National Biosciences, Plymouth, Minn.) was used to confirm the absence of self complementary regions within and between the primers or probes. When required, the primers contained inosines or degenerated nucleotides at one or more variable positions. Oligonucleotide primers and probes were synthesized on a model 394 DNA synthesizer (Perkin-Elmer Corp., Applied Biosystems Division, Mississauga, Ontario, Canada). Detection of the hybridization was performed with the DIG-labeled dUTP incorporated during the amplification with the *Staphylococcus*-specific PCR assay and hybridization signal was detected with a luminometer (Dynex Technologies) as described above in the section on luminescent detection of amplification products. Annexes XIV to XVII illustrate the internal probes which are more specific and/or have the best signal to background ratio.

PCR amplification. For all bacterial species, amplification was performed from purified genomic DNA or from a bacterial suspension whose turbidity was adjusted to that of a 0.5 McFarland standard, which corresponds to approximately 1.5×10^8 bacteria per ml. One nanogram of genomic DNA or 1 µl of the standardized bacterial suspension was transferred directly to a 19 µl PCR mixture. Each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.2 µM (each) of the two *Staphylococcus* genus-specific primers (TStaG-422 and TStaG-765, SEQ ID NO. 553 and 575), 200 µM (each) of the four deoxynucleoside triphosphates (Pharmacia Biotech), 3.3 µg/µl bovine serum albumin (BSA) (Sigma-Aldrich Canada Ltd, Oakville, Ontario, Canada), and 0.5 U *Taq* polymerase (Promega) coupled with *Taq*Start™ Antibody (Clontech). The PCR amplification and the agarose gel analysis of the amplified products were performed as previously described.

For determination of the sensitivities of the PCR assays, two-fold dilutions of purified genomic DNA were used to determine the minimal number of genome copies which can be detected.

Results

Amplifications with the *Staphylococcus*-specific PCR assay. The specificity of the assay was assessed by performing 30-cycle and 40-cycle PCR amplifications with the panel of gram-positive (47 species from 8 genera) and gram-negative (37 species from 22 genera) bacterial species listed in Table 11. The PCR assay was able to detect 27 of 27 staphylococcal species tested in both 30-cycle and 40-cycle regimens. For 30-cycle PCR, all bacterial species tested other than staphylococci were negative. For 40-cycle PCR, *Enterococcus faecalis, Lactobacillus acidophilus, Lactococcus lactis, Macrococcus caseolyticus, Streptocuccus agalactiae and S. mutans* were slightly positive for the *Staphylococcus*-specific PCR assay. The other species tested remained negative. Ubiquity tests performed on a collection of 295 clinical isolates provided by the microbiology laboratory of the Centre Hospitalier Universitaire de Québec, Pavillon Centre Hospitalier de l'Université Laval (CHUL) including *Staphylococcus aureus* (n=34), *S. auricularis* (n=2), *S. capitis* (n=19), *S. cohnii* (n=5), *S. epidermidis* (n=18), *S. haemolyticus* (n=21), *S. hominis* (n=73), *S. lugdunensis* (n=17), *S. saprophyticus* (n=6), *S. simulans* (n=3), *S. warneri* (n=32) and *Staphylococcus* spp. (n=65) showed a uniform amplification signal with the 30-cycle PCR assays and a perfect relation between the genotype and classical identification schemes.

The sensitivity of the *Staphylococcus*-specific assay with 30-cycle and 40-cycle PCR protocols was determined by using purified genomic DNA from the 11 staphylococcal species previously mentioned. For PCR with 30 cycles, a detection limit of 50 copies of genomic DNA was consistently obtained. In order to enhance the sensitivity of the assay, the number of cycles was increased. For 40 cycles PCR assays, the detection limit was lowered to a range of 5-10 genome copies, depending on the staphylococcal species tested.

Hybridization between *Staphylococcus*-specific 371-bp amplicon and species-specific internal probes. Inter-species polymorphism was sufficient to generate species-specific internal probes for each of the principal species involved in humans diseases, *S. aureus, S. epidermidis, S. haemolyticus, S. hominis* and *S. saprophyticus*. In order to verify the intra-specific sequence conservation of the nucleotide sequence, sequences comparisons were performed on the 371-bp amplicon from five unrelated ATCC and clinical strains for each of 5 staphylococcal species; *S. aureus, S.*

epidermidis, S. haemolyticus, S. hominis and *S. saprophyticus*. Results showed a high level of conservation of nucleotide sequence between different unrelated strains from the same species. This sequence information allowed the development of staphylococcal species identification assays using species-specific internal probes hybridizing to the 371-bp amplicon. In addition to the species-specific internal probes, the genus-specific internals probes were able to recognized most *Staphylococcus* species. These assays are specific and ubiquitous for those five staphylococcal species.

EXAMPLE 8:

Differentiating between the two closely related yeast species *Candida albicans* and *Candida dubliniensis*. It is often useful for the clinician to be able to differentiate between two very closely related species of microorganisms. *Candida albicans* is the most important cause of invasive human mycose. In the recent years, a very closely related species, *Candida dubliniensis*, was isolated in immunosuppressed patients. These two species are difficult to distinguish by classic biochemical methods. This example demonstrates the use of *tuf* sequences to differenciate *Candida albicans* and *Candida dubliniensis*. PCR primers TCal528 and TCal676 were selected for their ability to specifically amplify a *tuf* (elongation factor 1 alpha type) fragment from both species (see Annex XI for primer positions and previous patent application WO 98/20157 SEQ ID NO. 11-12). Within this *tuf* fragment, a region differentiating *C. albicans* and *C. dubliniensis* by two nucleotides was selected and used to design two internal probes (see Annex IV for probe design, SEQ ID NO. 577 and 578) specific for each species. Amplification of genomic DNA from *C. albicans* and *C. dubliniensis* was carried out using DIG-11-dUTP as described above in the section on luminescent detection of amplification products. Internal probes SEQ ID NO. 577 and 578 were immobilized on the bottom of individual microtiter plates and hybridization was carried out as described above in the above section on luminescent detection of amplification products. Luminometer data showed that the amplicon from *C. albicans* hybridized only to probe SEQ ID NO. 577 while the amplicon from *C. dubliniensis* hybridized only to probe SEQ ID NO. 578, thereby demonstrating that each probes were species-specific.

EXAMPLE 9:

Specific identification of *Entamoeba histolytica*. Upon analysis of *tuf* (elongation factor 1 alpha) sequence data, it was possible to find four regions where *Entamoeba histolytica* sequences remained conserved while other parasitical and eukaryotic species have diverged. Primers TEntG38 (SEQ ID NO. 703), TEntG442 (SEQ ID

NO. 704), TEntG534 (SEQ ID NO. 705), and TEntG768 (SEQ ID NO. 706) were designed so that Entg38 could be paired with the three other primers.On PTC-200 thermocyclers (MJ Research), the cycling conditions for initial sensitivity and specificity testing were 3 min. at 94°C for initial denaturation, thenforty cycles of two steps consisting of 1 second at 95°C and 30 seconds at 55°C, followed by terminal extension at 72°C for 2 minutes. Amplification was monitored on agarose gel electrophoresis by staining the amplified DNA with ethidium bromide. The three primer pairs could detect the equivalent of less than 200 *E. histolytica*genome copies. Specificity was tested using 0.5 ng of purified genomic DNA from a panel of microorganisms including *Babesia bovis, Babesia microtti, Candida albicans, Crithidia fasciculata, Leishmania major, Leishmania hertigi* and *Neospora caninum.* Only *E. histolytica* DNA could be amplified. Thereby suggesting that the assay was species-specific.

EXAMPLE 10:

Sensitive identification of *Chlamydia trachomatis*. Upon analysis of *tuf* sequence data, it was possible to find two regions where *Chlamydia trachomatis* sequences remained conserved while other species have diverged. Primers Ctr82 (SEQ ID NO. 554) and Ctr249 (SEQ ID NO. 555) were designed. With the PTC-200 thermocyclers (MJ Research), the optimal cycling conditions for maximum sensitivity and specificity were determined to be 3 min. at 94°C for initial denaturation, thenforty cycles of two steps consisting of 1 second at 95°C and 30 seconds at 60°C, followed by terminal extension at 72°C for 2 minutes. Amplification was monitored on agarose gel electrophoresis by staining the amplified DNA with ethidium bromide. The assay could detect the equivalent of 8 *C. trachomatis* genome copies. Specificity was tested on 0.1 ng of purified genomic DNA from a panel of microorganisms including 22 species commonly encountered in the vaginal flora (*Bacillus subtilis, Bacteroides fragilis, Candida albicans, Clostridium difficile, Corynebacterium cervicis, Corynebacterium urealyticum, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Fusobacterium nucleatum, Gardnerella vaginalis, Haemophilus influenzae, Klebsiella oxytoca, Lactobacillus acidophilus, Peptococcus niger, Peptostreptococcus prevotii, Porphyromonas asaccharolytica, Prevotella melaninogenica, Propionibacterium acnes, Staphylococcus aureus, Streptococcus acidominimus,* and *Streptococcus agalactiae*). Only *C. trachomatis* DNA could be amplified. Thereby suggesting that the assay was species-specific.

EXAMPLE 11:

Genus-specific identification of Enterococci. Upon analysis of *tuf* sequence data and comparison with the repertory of *tuf* sequences, it was possible to find two regions where *Enterococcus* sequences remained conserved while other genera have diverged (Annex XVII). Primers Encg 313 (SEQ ID NO. 656) and Encg 596 (SEQ ID NO. 657) were tested for their specificity by using purified genomic DNA from a panel of bacteria listed in Table 10. Using the PTC-200 thermocyclers (MJ Research), the optimal cycling conditions for maximum sensitivity and specificity were determined to be 3 min. at 94°C for initial denaturation, then forty cycles of two steps consisting of 1 second at 95°C and 30 seconds at 62°C, followed by terminal extension at 72°C for 2 minutes. Amplification was monitored on agarose gel electrophoresis by staining the amplified DNA with ethidium bromide. The 17 enterococcal species listed in Table 10 were all amplified. The only other species amplified were *Abiotrophia adiacens* and *Gemella haemolysans*, two Gram positive species. The sequence variation within the 306 bp amplicon is sufficient so that nested PCR or internal probes could be used to speciate the amplicon and differenciate enterococci from *Abiotrophia adiacens* and *Gemella haemolysans*. Sensitivity tested on several strains of the clinically important species *E. casseliflavus, E. faecium, E. faecalis, E. flavescens* and *E. gallinarum* ranged from 1 to 8 genome copies. A mismatch for all enterococcal species (except *E. faecalis* and *E. solitarius*) was introduced inadvertantly in position 12 (C instead of G) of primer SEQ ID NO. 657. Apparently, this mismatch did not significantly impaired the primer pair's sensitivity and specificity. Because of the known tolerance of PCR primers to mismatches in their middle, non-mismatch primer pair SEQ ID NO. 656 + SEQ ID NO. 271 should give specificity and sensitivity results similar to SEQ ID NO. 656 + SEQ ID NO. 657.

EXAMPLE 12:

Identification of the major bacterial platelets contaminants using *tuf* sequences in a multiplex test. Blood platelets preparations need to be monitored for bacterial contaminations. The *tuf* sequences of 17 important bacterial contaminants of platelets were aligned. As shown in Annex XIX, analysis of these sequences allowed the design PCR primers. Since in the case of platelet contamination, detecting all species, not just the more frequently encountered ones is desirable, perfect specificity of primers was not an issue in the design. However, sensitivity is important. That is why, to avoid having to put too much degeneracy, only the most frequent contaminants were included in primer design, knowing that the selected primers would anyway be able to amplify more species than the 17 used in the design. Oligonucleotide sequences which are conserved in these 17 major bacterial contaminants of platelets were chosen (oligos Tplaq 769 and Tplaq 991, respectively SEQ ID NO. 636 and 637) thereby permitting the detection of these bacterial species.

However, sensitivity was a bit deficient with staphylococci. To ensure maximal sensitivity in the detection of all the more frequent bacterial contaminants, a multiplex assay also including oligonucleotide primers targetting the *Staphylococcus* genera (oligos Stag 422, SEQ ID NO. 553; and Stag 765, SEQ ID NO. 575) was developed.

The primer pairs, oligos Tplaq 769 (SEQ ID NO. 636) and Tplaq 991 (SEQ ID NO. 637) that give an amplification product of 245 pb and oligos TStaG 422 (SEQ ID NO. 553) and TStaG 765 (SEQ ID NO. 575) that give an amplification product of 368 pb, were used simultaneously in the multiplex PCR assay. Real-time detection of these PCR products was made on the LightCycler thermocycler (Idaho Technology) using SYBR® Green I (Molecular Probe Inc.). SYBR®Green I is a fluorescent dye that binds specifically to double-stranded DNA. It thus binds to DNA products as they are synthesized. The measure of SYBR®Green I fluorescence at the end of each elongation cycle indicates the amount of DNA duplex generated by specific DNA fragment amplification and primer-dimer formation.

Real-time detection of PCR products with the LightCycler was carried out using 1.0 mM of both Tplaq primers (SEQ ID NO. 636-637) and 0.4 mM of both TStaG primers (SEQ ID NO. 553 and 575), 2.5 mM $MgCl_2$, BSA 500 mg/ml , dNTP 0.2 mM (Pharmacia), 10X PCR reaction buffer (Boerhinger Mannheim) and Taq DNA polymerase (Boerhinger Mannheim) 0.025 U/ml combined with TaqStart 5 ng/ml (Clontech), and 0.7 ml of genomic DNA sample in a final volume of 7 ml. The optimal cycling conditions for maximum sensitivity were 1 minute at 94°C for initial denaturation, then forty-five cycles of three steps consisting of 0 second at 95°C, 5 seconds at 60°C and 9 seconds at 72°C. Amplification was monitored during each elongation cycle by measuring the level of SYBR®Green I. However, real analysis takes place after PCR. Melting curves are done for each sample and transformation of melting peak allows determination of Tm. Thus primer-dimer and specific PCR product are discriminated. With this assay, all prominent bacterial contaminants of platelets listed in Annex XIX were detected. Sensitivity tests were performed on the 9 most frequent bacterial contaminants of platelets. The detection limit was less than 20 genome copies for *E. cloacae*, *B. cereus*, *S. choleraesuis* and *S. marcescens*; less than 15 genome copies for *P. aeruginosa*; and 2 to 3 copies were detected for *S. aureus*, *S. epidermidis*, *E. coli* and *K. pneumoniae*. Further refinements of assay conditions should increase sensitivity levels.

EXAMPLE 13:

The resolving power of the *tuf* and *atpD* sequences databases is comparable to the gold standard biochemical methods for bacterial identification. The present gold standard for bacterial identification is mainly based on key morphological traits and batteries of biochemical tests. Here we demonstrate that the use of *tuf* and *atpD* sequences combined with simple phylogenetic analysis of databases formed by these sequences is comparable to the gold standard. In the process of acquiring data for the *tuf* sequences, we sequenced the *tuf* gene of a strain that was given to us labelled as *Staphylococcus hominis* ATCC 35982. That *tuf* sequence (SEQ ID NO: 192) was incorporated into the *tuf* sequences database and subjected to a basic phylogenic analysis using the Pileup command from version 10 of the GCG package (Genetics Computer Group, inc.). This analysis indicated that SEQ ID NO: 192 is not associated with other *S. hominis* strains but rather with the *S. warneri* strains. The ATCC 35982 strain was sent to the reference laboratory of Laboratoire de Santé publique du Québec (LSPQ). They used the classic gold standard identification scheme for staphylococci (Kloos and Schleifer, 1975., J. Clin. Microbiol.**1**:82-88). Their results shown that although the colonial morphology could correspond to*S. hominis*, the more precise biochemical assays did not. These assays included discriminant mannitol, mannose and ribose acidification tests as well as rapid and dense growth in deep thioglycolate agar. The LSPQ report identified strain ATCC 35982 as *S. warneri* which confirms our database analysis. The same thing happened for *S. warneri* (SEQ ID NO: 187) which had initially been identified as *S. haemolyticus* by a routine clinical laboratory using a low resolving power automated system (MicroScan, AutoScan-4™). Again, the *tuf* and LSPQ analysis agreed on its identification as *S. warneri*. In numerous other instances, in the course of acquiring *tuf* and *atpD* sequence data from various species and genera, analysis of our *tuf* and/or *atpD* sequence databases permitted the exact identification of mislabelled or erroneously identified strains. These results clearly demonstrate the usefulness and the high resolving power of our sequence-based identification assays using the*tuf* and *atpD* sequences databases.

Example 14:

Detection of group B streptococci in clinical specimens.

Introduction

Streptococcus agalactiae, the group B streptococcus (GBS), is responsible for a severe illness affecting neonate infants. The bacterium is passed from the healthy carrier mother to the baby during delivery. To prevent this infection, it is recommended to treat expectant mothers susceptible of carrying GBS in their anovaginal flora. Carrier status is often a transient condition and rigorous monitoring

requires cultures and classic bacterial identification weeks before delivery. To improve the diagnostic and identification of GBS we developped a rapid, specific and sensitive PCR test fast enought to be performed right at delivery.

Materials and Methods

GBS Clinical Specimens. A total of 66 duplicate anovaginal swabs were collected from 41 consenting pregnant women admitted for delivery at the Centre Hospitalier Universitaire de Québec, Pavillon Saint-François d'Assise following the CDC recommendations. The samples were obtained either before or after rupture of membranes. The swab samples were tested at the Centre de Recherche en Infectiologie de l'Université Laval within 24 hours of collection. Upon receipt, one swab was cut and then the tip of the swab was added to GNS selective broth for identification of group B streptococci (GBS) by the standard culture methods recommended by the Center for Diseases Control. The other swab was processed following the instruction of the IDI DNA extraction kit (Infectio Diagnotics (IDI) Inc.) prior to PCR amplification.

Oligonucleotides. PCR primers, Tsag340 (SEQ ID NO. 549) and Tsag552 (SEQ ID NO. 550) complementary to the regions of the tuf gene unique for GBS were designed based upon multiple sequence alignment using our repertory of tuf sequences. Oligo primer analysis software (version 5.0) (National Biosciences) was used to analyse primers annealing temperature, secondary structure potential as well as mispriming and dimerization potential. The primers were synthesized using a model 391 DNA synthesizer (Perkin-Elmer).

A pair of fluorescently labeled adjacent hybridization probes Sag465-F (SEQ ID NO. 583) and Sag436-C (SEQ ID NO. 582) were synthesized and purified by Operon Technologies. They were designed to meet the recommendations of the manufacturer (Idaho Technology) and based upon multiple sequence alignment analysis using our repertory of *tuf* sequences to be specific and ubiquitous for GBS. These adjacent probes, which are separated by one nucleotide, allowing fluorescence resonance energy transfer (FRET) to generate an increased fluorescence signal when both hybridized simultaneously to their target sequences. The probes Sag465-F was labeled with FITC in 3 prime while Sag436-C was labeled with Cy5 in 5 prime. The Cy5-labeled probes contained a 3'-blocking phosphate group to prevent extension of the probes during the PCR reactions.

PCR Amplification. Conventional amplifications were performed either from 2 µl of a purified genomic DNA preparation or cell lysates of anovaginal specimens. The 20-µl PCR mixture contained 0.4 µM each GBS-specific primer (Sag465-F/Sag436), 200 µM each deoxyribonucleotide (Pharmacia Biotech), 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 2.5 mM $MgCl_2$, 3.3 mgml bovine serum albumin (BSA) (Sigma), and 0.5 U of *Taq* polymerase (Promega) combined with the

TaqStart antibody (Clontech). The TaqStart antibody, which is a neutralizing monoclonal antibody of *Taq* DNA polymerase, was added to all PCR reactions to enhance the efficiency of the amplification. The PCR mixtures were subjected to thermal cycling (3 min at 95°C and then 40 cycles of 1 s at 95°C, and 30 s at 62°C with a 2-min final extension at 72°C) with a PTC-200 DNA Engine thermocycler (MJ research). The PCR-amplified reaction mixture was resolved by agarose gel electrophoresis.

The LightCycler™ PCR amplifications were performed with 1 μl of the same preparation as described above. The 10-μl amplification mixture consisted of 0.4 μM each GBS-specific primer (Sag465-F/Sag436), 200 μM each dNTP, 0.2 μM each fluorescently labeled probe (Sag465-F and Sag436-C), 300μg/ml BSA (Sigma), and 1 μl of 10x PC2 buffer (containing 50 mM Tris-HCl (pH 9.1), 16 mM ammonium sulfate, 3.5 mM Mg^{2+}, and 150 μg/ml BSA) and 0.5 U KlenTaq1™ (AB Peptides) coupled with TaqStart™ antibody (Clontech). KlenTaq1™ is a highly active and more heat-stable DNA polymerase without 5'-exonuclease activity. This prevents hydrolysis of hybridized probes by the 5' to 3' exonuclease activity. A volume of 7μl of the PCR mixture was transferred into a composite capillary tube (Idaho Technology). The tubes were then centrifuged to move the reaction mixture to the tips of the capillaries and then cleaned with optical-grade methanol. Subsequently the capillaries were loaded into the carousel of a LC32 LightCycler™ (Idaho Technology), an instruments that combine rapid-cycle PCR with fluorescence analysis for continuous monitoring during amplification. The PCR reaction mixtures were subjected to a denaturation step at 94°C for 3 min followed by 45 cycles of 0 s at 94°C, 20 s at 64°C and 10 s at 72°C with a temperature transition rate of 20°C/s. Fluorescence signals were obtained at each cycle by sequentially positioning each capillary on the carousel at the focus of optics affiliated to the built-in fluorimeter for 100 millisecond. Complete amplification and analysis required about 35 min.

Specificity And Sensitivity Tests. The specificity of the conventional and LightCycler™ PCR assay was verified by using purified genomic DNA (0.1 ng/reaction) from a battery of ATCC reference strains representing 35 clinically relevent gram-positive species (*Abiotrophia defectiva* ATCC 49176, *Bifidobacterium breve* ATCC 15700, *Clostridium difficile* ATCC 9689, *Corynebacterium urealyticum* ATCC 43042, *Enterococcus casseliflavus* ATCC 25788, *Enterococcus durans* ATCC 19432, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* ATCC 19434, *Enterococcus gallinarum* ATCC 49573, *Enterococcus raffinosus* ATCC 49427, *Lactobacillus reuteri* ATCC 23273, *Lactococcus lactis* ATCC 19435, *Listeria monocytogenes* ATCC 15313, *Peptococcus niger* ATCC 27731, *Peptostreptococcus anaerobius* ATCC 27337, *Peptostreptococcus prevotii* ATCC 9321, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 14990, *Staphylococcus haemolyticus* ATCC 29970, *Staphylococcus saprophyticus* ATCC 15305,

Streptococcus agalactiae ATCC 27591, *Streptococcus anginosus* ATCC 33397, *Streptococcus bovis* ATCC 33317, *Streptococcus constellatus* ATCC 27823, *Streptococcus dysgalactiae* ATCC 43078, *Streptococcus gordonii* ATCC 10558, *Streptococcus mitis* ATCC 33399, *Streptococcus mutans* ATCC 25175, *Streptococcus oralis* ATCC 35037, *Streptococcus parauberis* ATCC 6631, *Streptococcus pneumoniae* ATCC 6303, *Streptococcus pyogenes* ATCC 19615, *Streptococcus salivarius* ATCC 7073, *Streptococcus sanguis* ATCC 10556, *Streptococcus uberis* ATCC 19436). These microbial species included 15 species of streptococci and many members of the normal vaginal and anal floras. In addition, 40 GBS isolates of human origins confirm by Latex agglutination test (Streptex, Murex) were also used to evaluate the ubiquity of the assay.

For determination of the sensitivities (i.e., the minimal number of genome copies that could be detected) for conventional and LightCycler™ PCR assays, serial 10-fold or 2-fold dilutions of purified genomic DNA from 5 GBS ATCC strains were used.

Results

Evaluation of the GBS-specific conventional and LightCycler™ PCR assay. The specificity of the two assays demonstrated that only DNAs from GBS strains could be amplified. Both PCR assays did not amplify DNAs from any other bacterial species tested including 14 streptococcal species other than GBS as well as phylogenetically related species belonging to the genus *Enterococcus*, *Peptostreptococcus* and *Lactococcus*. Important members of the vaginal or anal flora, including coagulase-negative staphylococci, *Lactobacillus* spp., and *Bacteriodes* spp. were also negative with the GBS-specific PCR assay. The LightCycler™ PCR assays detected only GBS DNA by producing an increased fluorescence signal which was interpreted as a positive PCR result. Both PCR methods were able to amplify all of 40 GBS clinical isolates, showing a perfect correlation with the phenotypic identification methods.

The sensitivity of the assay was determined by using purified genomic DNA from the 5 ATCC strains of GBS. The detection limit for all of these 5 strains was one genome copy of GBS. The detection limit of the assay with the LightCycler™ was 3.5 fg of genomic DNA (corresponding to 1-2 genome copies of GBS). These results confirmed the high sensitivity of our GBS-specific PCR assay.

Direct Detection of GBS from anovaginal specimens. Among 66 anovaginal specimens tested, 12 were positive for GBS by culture. 11 of them were also identified by both PCR assays. The sensitivity of both PCR methods with vaginal/anal specimens for identifying colonization status in pregnant women at delivery was 91.7% when compared to culture results. Specificity and positive predictive value were both 100% and negative predictive value was 97.8%. The time

for obtaining results was approximately 50 min for LightCycler™ PCR, approximately 100 min for conventional PCR and 48 hours for culture.

Conclusion

We have developed two PCR assays (conventional and LightCycler™) for the detection of GBS which are specific (i.e., no amplification of DNA from a variety of bacterial species other than GBS) and sensitive (i.e., able to detect around 1 genome copy for several reference ATCC strains of GBS). Both PCR assays are able to detect GBS directly from anovaginal specimens in a very short turnaround time. Using the real-time PCR assay on LightCycler™, we can detect GBS carriage in pregnant women at delivery within 50 minutes.

This invention has been described herein above, and it is readily apparent that modifications can be made thereto without departing from the spirit of this invention. These modifications are under the scope of this invention, as defined in the appended claims.

Table 1. Distribution (%) of nosocomial pathogens for various human infections in USA (1990-1992)[1].

Pathogen	UTI[2]	SSI[3]	BSI[4]	Pneumonia	CSF[5]
Escherichia coli	27	9	5	4	2
Staphylococcus aureus	2	21	17	21	2
Staphylococcus epidermidis	2	6	20	0	1
Enterococcus faecalis	16	12	9	2	0
Enterococcus faecium	1	1	0	0	0
Pseudomonas aeruginosa	12	9	3	18	0
Klebsiella pneumoniae	7	3	4	9	0
Proteus mirabilis	5	3	1	2	0
Streptococcus pneumoniae	0	0	3	1	18
Group B *Streptococci*	1	1	2	1	6
Other *Streptococci*	3	5	2	1	3
Haemophilus influenzae	0	0	0	6	45
Neisseria meningitidis	0	0	0	0	14
Listeria monocytogenes	0	0	0	0	3
Other *Enterococci*	1	1	0	0	0
Other *Staphylococci*	2	8	13	2	0
Candida albicans	9	3	5	5	0
Other *Candida*	2	1	3	1	0
Enterobacter spp.	5	7	4	12	2
Acinetobacter spp.	1	1	2	4	2
Citrobacter spp.	2	1	1	1	0
Serratia marcescens	1	1	1	3	1
Other *Klebsiella*	1	1	1	2	1
Others	0	6	4	5	0

[1] Data recorded by the National Nosocomial Infections Surveillance (NNIS) from 80 hospitals (Emori and Gaynes, 1993, *Clin. Microbiol. Rev.*, 6:428-442).

[2] Urinary tract infection.

[3] Surgical site infection.

[4] Bloodstream infection.

[5] Cerebrospinal fluid.

Table 2. Distribution (%) of blo dstr am infecti n pathogens In Quebec (1995), Canada (1992), UK (1969-1988) and USA (1990-1992).

Organism	Quebec[1]	Canada[2]	UK[3]		USA[4]
			Community-acquired	Hospital-acquired	Hospital-acquired
E. coli	15.6	53.8	24.8	20.3	5.0
S. epidermidis and other CoNS[5]	25.8	-	0.5	7.2	31.0
S. aureus	9.6	-	9.7	19.4	16.0
S. pneumoniae	6.3	-	22.5	2.2	-
E. faecalis	3.0	-	1.0	4.2	-
E. faecium	2.6	-	0.2	0.5	-
Enterococcus spp.	-	-	-	-	9.0
H. influenzae	1.5	-	3.4	0.4	-
P. aeruginosa	1.5	8.2	1.0	8.2	3.0
K. pneumoniae	3.0	11.2	3.0	9.2	4.0
P. mirabilis	-	3.9	2.8	5.3	1.0
S. pyogenes	-	-	1.9	0.9	-
Enterobacter spp.	4.1	5.5	0.5	2.3	4.0
Candida spp.	8.5	-	-	1.0	8.0
Others	18.5	17.4	28.7	18.9	19.0

[1] Data obtained for 270 isolates collected at the Centre Hospitalier de l'Université Laval (CHUL) during a 5 month period (May to October 1995).

[2] Data from 10 hospitals throughout Canada representing 941 gram-negative isolates. (Chamberland *et al.*, 1992, *Clin. Infect. Dis.*, **15**:615-628).

[3] Data from a 20-year study (1969-1988) for nearly 4000 isolates. (Eykyn *et al.*, 1990, *J. Antimicrob. Chemother.*, Suppl. C, **25**:41-58).

[4] Data recorded by the National Nosocomial Infections Surveillance (NNIS) from 80 hospitals (Emori and Gaynes, 1993, *Clin. Microbiol. Rev.*, **6**:428-442).

[5] Coagulase-negative staphylococci.

Table 3. Distribution of positive and negative clinical specimens tested at the microbiology laboratory of the CHUL (February 1994 – January 1995).

Clinical specimens and/or sites	No. of samples tested (%)	% of positive specimens	% of negative specimens
Urine	17,981 (54.5)	19.4	80.6
Blood culture/marrow	10,010 (30.4)	6.9	93.1
Sputum	1,266 (3.8)	68.4	31.6
Superficial pus	1,136 (3.5)	72.3	27.7
Cerebrospinal fluid	553 (1.7)	1.0	99.0
Synovial fluid	523 (1.6)	2.7	97.3
Respiratory tract	502 (1.5)	56.6	43.4
Deep pus	473 (1.4)	56.8	43.2
Ears	289 (0.9)	47.1	52.9
Pleural and pericardial fluid	132 (0.4)	1.0	99.0
Peritoneal fluid	101(0.3)	28.6	71.4
Total:	**32,966 (100.0)**	**20.0**	**80.0**

Table 4. **Non limitating example of microbial species for which *atpD* and/or *tuf* and/or *recA* sequences are used in the present invention**

Bacterial species

Achromobacter xylosoxidans subsp. *denitrificans*
Acetobacterium woodi
Acetobacter aceti
Acetobacter altoacetigenes
Acetobacter polyoxogenes
Acholeplasma laidlawii
Acidiphilum facilis
Acinetobacter baumannii
Acinetobacter calcoaceticus
Acinetobacter lwoffii
Actinomyces meyeri
Aerococcus viridans
Aeromonas salmonicida
Agrobacterium tumefaciens
Alcaligenes faecalis
Allochromatium vinosum
Anabaena variabilis
Anacystis nidulans
Anaerorhabdus furcosus
Aquifex aeolicus
Aquifex pyrophilus
Azotobacter vinelandii
Bacillus anthracis
Bacillus caldotenax
Bacillus cereus
Bacillus firmus
Bacillus halodurans
Bacillus megaterium
Bacillus stearothermophilus
Bacillus subtilis
Bacteroides distasonis
Bacteroides fragilis
Bacteroides ovatus
Bacteroides vulgatus
Bartonella henselae
Bifidobacterium adolescentis
Bifidobacterium breve
Bifidobacterium dentium
Bifidobacterium longum
Blastochloris viridis
Borrelia burgdorferi
Bordetella pertussis
Bordetella bronchiseptica
Branhamella catarrhalis
Brucella abortus
Brevibacterium linens
Brevibacterium flavum
Buchnera aphidicola
Burkholderia cepacia
Burkholderia mallei
Burkholderia pseudomallei
Campylobacter jejuni
Cedecea davisae
Cedecea lapagei
Cedecea neteri
Chlamydia pneumoniae
Chlamydia psittaci
Chlamydia trachomatis
Chlorobium vibrioforme
Chloroflexus aurantiacus
Chryseobacterium meningosepticum
Citrobacter amalonaticus
Citrobacter braakii
Citrobacter farmeri
Citrobacter freundii
Citrobacter koseri
Citrobacter sedlakii
Citrobacter werkmanii
Citrobacter youngae
Clostridium acetobutylicum
Clostridium beijerincki
Clostridium bifermentans
Clostridium botulinum
Clostridium difficile
Clostridium innocuum
Clostridium histolyticum
Clostridium novyi
Clostridium septicum
Clostridium perfringens
Clostridium ramosum
Clostridium sordellii
Clostridium tertium
Clostridium tetani
Comamonas acidovorans
Corynebacterium bovis
Corynebacterium cervicis
Corynebacterium diphtheriae
Corynebacterium flavescens
Corynebacterium glutamicum
Corynebacterium kutscheri
Corynebacterium minutissimum
Corynebacterium mycetoides
Corynebacterium pseudodiphtheriticum
Corynebacterium pseudogenitalium
Corynebacterium pseudotuberculosis
Corynebacterium renale
Corynebacterium ulcerans
Corynebacterium urealyticum
Corynebacterium xerosis
Coxiella burnetii
Cytophaga lytica
Deinococcus radiodurans
Deinonema spp.
Edwardsiella hoshinae
Edwardsiella tarda
Ehrlichia canis
Ehrlichia risticii
Eikenella corrodens
Enterobacter aerogenes
Enterobacter agglomerans
Enterobacter amnigenus
Enterobacter asburiae
Enterobacter cancerogenus
Enterobacter cloacae

Table 4. Non limitating example of microbial sp cies for which *atpD* and/or *tuf* and/or *recA* s quenc s are used in the present inv nti n (c ntinued)

Bacterial species (continued)

Enterobacter gergoviae
Enterobacter hormaechei
Enterobacter sakazakii
Enterococcus avium
Enterococcus casseliflavus
Enterococcus cecorum
Enterococcus dispar
Enterococcus durans
Enterococcus faecalis
Enterococcus faecium
Enterococcus flavescens
Enterococcus gallinarum
Enterococcus hirae
Enterococcus malodoratus
Enterococcus mundtii
Enterococcus pseudoavium
Enterococcus raffinosus
Enterococcus saccharolyticus
Enterococcus solitarius
Enterococcus sulfureus
Erwinia carotovora
Escherichia coli
Escherichia fergusonii
Escherichia hermannii
Escherichia vulneris
Eubacterium lentum
Eubacterium nodatum
Ewingella americana
Francisella tularensis
Frankia alni
Fervidobacterium islandicum
Fibrobacter succinogenes
Flavobacterium ferrigeneum
Flexistipes sinusarabici
Fusobacterium gonidiaformans
Fusobacterium necrophorum subsp. *necrophorum*
Fusobacterium nucleatum subsp. *polymorphum*
Gardnerella vaginalis
Gemella haemolysans
Gemella morbillorum
Gloeobacter violaceus
Gloeothece spp.
Gluconobacter oxydans
Haemophilus actinomycetemcomitans
Haemophilus aphrophilus
Haemophilus ducreyi
Haemophilus haemolyticus
Haemophilus influenzae
Haemophilus parahaemolyticus
Haemophilus parainfluenzae
Haemophilus paraphrophilus
Haemophilus segnis
Hafnia alvei
Haloarcula marismortui
Halobacterium salinarum
Haloferax volcanii
Helicobacter pylori
Herpetoshiphon aurantiacus
Kingella kingae
Klebsiella ornithinolytica
Klebsiella oxytoca
Klebsiella planticola
Klebsiella pneumoniae subsp. *ozaenae*
Klebsiella pneumoniae subsp. *pneumoniae*
Klebsiella pneumoniae subsp. *rhinoscleromatis*
Kluyvera ascorbata
Kluyvera cryocrescens
Kluyvera georgiana
Lactobacillus acidophilus
Lactobacillus garvieae
Lactobacillus paracasei
Lactobacillus casei subsp. *casei*
Lactococcus lactis subsp. *lactis*
Leclercia adecarboxylata
Legionella micdadei
Legionella pneumophila subsp. *pneumophila*
Leminorella grimontii
Leminorella richardii
Leptospira biflexa
Leptospira interrogans
Listeria monocytogenes
Magnetospirillum magnetotacticum
Megamonas hypermegale
Methanobacterium thermoautotrophicum
Methanococcus jannaschii
Methanococcus vannielii
Methanosarcina barkeri
Methanosarcina jannaschii
Methylobacillus flagellatum
Methylomonas clara
Micrococcus luteus
Micrococcus lylae
Mitsuokella multacida
Mobiluncus curtisii subsp. *holmesii*
Moellerella thermoacetica
Moellerella wisconsensis
Moraxella osloensis
Morganella morganii subsp. *morganii*
Mycobacterium avium
Mycobacterium bovis
Mycobacterium leprae
Mycobacterium tuberculosis
Mycoplasma capricolum
Mycoplasma gallisepticum
Mycoplasma genitalium
Mycoplasma hominis
Mycoplasma pirum
Mycoplasma mycetoides
Mycoplasma pneumoniae
Mycoplasma pulmonis
Mycoplasma salivarium
Myxococcus xanthus
Neisseria animalis
Neisseria canis

Table 4. Non limiting example f microbial species f r which *atpD* and/or *tuf* and/or *recA* sequenc s ar used in the present inventi n (continued)

Bacterial species (continued)

Neisseria cinerea
Neisseria cuniculi
Neisseria elongata subsp. *elongata*
Neisseria elongata subsp. *intermedia*
Neisseria flavescens
Neisseria gonorrhoeae
Neisseria lactamica
Neisseria meningitidis
Neisseria mucosa
Neisseria perflava
Neisseria pharyngis
Neisseria polysaccharea
Neisseria sicca
Neisseria subflava
Neisseria weaveri
Ochrobactrum anthropi
Pantoea agglomerans
Pantoea dispersa
Paracoccus denitrificans
Pasteurella multocida
Pectinatus frisingensis
Peptococcus niger
Peptostreptococcus anaerobius
Peptostreptococcus asaccharolyticus
Peptostreptococcus prevotii
Phormidium ectocarpi
Pirellula marina
Planobispora rosea
Plectonema boryanum
Porphyromonas asaccharolytica
Porphyromonas gingivalis
Pragia fontium
Prevotella melaninogenica
Prevotella oralis
Prevotella ruminocola
Prochlorothrix hollandica
Propionibacterium acnes
Propionigenium modestum
Proteus mirabilis
Proteus penneri
Proteus vulgaris
Providencia alcalifaciens
Providencia rettgeri
Providencia rustigianii
Providencia stuartii
Pseudomonas aeruginosa
Pseudomonas fluorescens
Pseudomonas stutzeri
Psychrobacter phenylpyruvicus
Rahnella aquatilis
Rickettsia prowazekii
Rhodobacter capsulatus
Rhodobacter sphaerolides
Rhodospirillum rubrum
Ruminococcus albus
Salmonella bongori
Salmonella choleraesuis subsp. *arizonae*
Salmonella choleraesuis subsp *choleraesuis*
Salmonella choleraesuis subsp. *diarizonae*
Salmonella choleraesuis subsp. *houtenae*
Salmonella choleraesuis [illegible]
Salmonella choleraesuis subsp. *salamae*
Serpulina hyodysenteriae
Serratia ficaria
Serratia fonticola
Serratia grimesii
Serratia liquefaciens
Serratia marcescens
Serratia odorifera
Serratia plymuthica
Serratia rubidaea
Shewanella putida
Shewanella putrefaciens
Shigella boydii
Shigella dysenteriae
Shigella flexneri
Shigella sonnei
Spirochaeta aurantia
Staphylococcus aureus
Staphylococcus auricularis
Staphylococcus capitis subsp. *capitis*
Staphylococcus caseolyticus
Staphylococcus cohnii
Staphylococcus epidermidis
Staphylococcus haemolyticus
Staphylococcus hominis
Staphylococcus lugdunensis
Staphylococcus saprophyticus
Staphylococcus sciuri subsp. *sciuri*
Staphylococcus simulans
Staphylococcus warneri
Stigmatella aurantiaca
Stenotrophomonas maltophilia
Streptococcus acidominimus
Streptococcus agalactiae
Streptococcus anginosus
Streptococcus bovis
Streptococcus cricetus
Streptococcus cristatus
Streptococcus downei
Streptococcus dysgalactiae
Streptococcus equi subsp. *equi*
Streptococcus ferus
Streptococcus gordonii
Streptococcus macacae
Streptococcus mutans
Streptococcus oralis
Streptococcus parasanguinis
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus ratti
Streptococcus salivarius
Streptococcus salivarius subsp. *thermophilus*
Streptococcus sanguinis

Table 4. Non limiting example of microbial species for which *atpD* and/or *tuf* and/or *recA* sequences are used in the present invention (continued)

Bacterial species (continued)

Streptococcus sobrinus
Streptococcus suis
Streptococcus vestibularis
Streptomyces anbofaciens
Streptomyces aureofaciens
Streptomyces cinnamoneus
Streptomyces coelicolor
Streptomyces collinus
Streptomyces lividans
Streptomyces ramocissimus
Streptomyces rimosus
Streptomyces venezuelae
Synechococcus spp.
Synechocystis spp.
Tatumella ptyseos
Taxeobacter occealus
Thermoplasma acidophilum
Thermotoga maritima
Thermus aquaticus
Thermus thermophilus
Thiobacillus cuprinus
Thiobacillus ferrooxydans
Trabulsiella guamensis
Treponema pallidum *Streptococcus uberis*
Ureaplasma urealyticum
Veillonella parvula
Vibrio alginolyticus
Vibrio anguillarum
Vibrio cholerae
Wolinella succinogenes
Xanthomonas citri
Xanthomonas oryzae
Xenorhabdus bovieni
Xenorhabdus nematophilus
Yersinia bercovieri
Yersinia enterocolitica
Yersinia frederikensii
Yersinia intermedia
Yersinia pestis
Yersinia pseudotuberculosis
Yersinia rohdei
Yokenella regensburgei
Zoogloea ramigera

Fungal species

Absidia corymbifera
Absidia glauca
Alternaria alternata
Arxula adeninivorans
Aspergillus oryzae
Aspergillus flavus
Aspergillus fumigatus
Aspergillus niger
Aureobasidium pullulans
Bipolaris hawaiiensis
Blastoschizomyces capitatus
Candida albicans
Candida catenulata
Candida dubliniensis
Candida famata
Candida glabrata
Candida guilliermondii
Candida haemulonii
Candida inconspicua
Candida kefyr
Candida krusei
Candida lambica
Candida lusitaniae
Candida norvegensis
Candida parapsilosis
Candida rugosa
Candida sphaerica
Candida tropicalis
Candida utilis
Candida viswanathii
Candida zeylanoides
Cladophialophora carrionii
Coccidioides immitis
Coprinus cinereus
Cryptococcus albidus
Cryptococcus neoformans
Cunninghamella bertholletiae
Curvularia lunata
Emericella nidulans
Exophiala jeanselmei
Eremothecium gossypii
Fonsecaea pedrosoi
Fusarium oxysporum
Geotrichum spp.
Histoplasma capsulatum
Issatchenkia orientalis kudrjanzev
Kluyveromyces lactis
Malassezia furfur
Malassezia pachydermatis
Malbranchea filamentosa
Metschnikowia pulcherrima
Microsporum audouinii
Mucor circinelloides
Neurospora crassa
Paecilomyces lilacinus
Paracoccidioides brasiliensis
Penicillium marneffei
Phialaphora verrucosa
Pichia anomala
Piedrala hortai
Podospora anserina
Puccinia graminis
Pseudallescheria boydii
Rhizomucor racemosus

Table 4. Non limitating example of microbial species for which *atpD* and/or *tuf* and/or *recA* sequences are used in the present invention (continued)

Fungal species (continued)

Rhizopus oryzae
Rhodotorula minuta
Saccharomyces cerevisiae
Saksenaea vasiformis
Schizosaccharomyces pombe
Scopulariopsis koningii
Sporobolomyces salmonicolor
Sporothrix schenckii
Stephanoascus ciferrii
Syncephalastrum racemosum
Trichoderma reesei *Rhodotorula mucilaginosa*
Trichophyton mentagrophytes
Trichophyton tonsurans
Trichosporon cutaneum
Ustilago maydis
Wangiella dermatitidis
Yarrowia lipolytica

Parasitical species

Babesia bigemina
Babesia bovis
Babesia microtti
Blastocystis hominis
Crithidia fasciculata
Cryptosporidium parvum
Entamoeba histolytica
Giardia lamblia
Kentrophoros spp.
Leishmania aethiopica
Leishmania amazonensis
Leishmania braziliensis
Leishmania donovani subsp. *donovani*
Leishmania donovani subsp. *infantum*
Leishmania enriettii
Leishmania gerbilli
Leishmania guyanensis
Leishmania hertigi subsp. *hertigi*
Leishmania major
Leishmania mexicana
Leishmania tarentolae
Leishmania tropica
Neospora caninum
Onchocerca volvulus
Plasmodium berghei
Plasmodium falciparum
Plasmodium knowlesi
Porphyra purpurea
Toxoplasma gondii
Treponema pallidum
Trichomonas vaginalis
Trypanosoma brucei subsp. *brucei*
Trypanosoma congolense
Trypanosoma cruzi

Table 5. Antibiotic resistance genes selected for diagnostic purposes

Genes	Antibiotics	Bacteria[1]	ACCESSION NO.	SEQ ID NO. (genes)
aac(3)-Ib [2]	Aminoglycosides	*Enterobacteriaceae Pseudomonads*	L06157	
aac(3)-IIb [2]	Aminoglycosides	*Enterobacteriaceae, Pseudomonads*	M97172	
aac(3)-IVa [2]	Aminoglycosides	*Enterobacteriaceae*	X01385	
aac(3)-VIa [2]	Aminoglycosides	*Enterobacteriaceae, Pseudomonads*	M88012	
aac(2')-1a [2]	Aminoglycosides	*Enterobacteriaceae, Pseudomonads*	X04555	
aac(6')-aph(2") [2]	Aminoglycosides	*Enterococcus* spp., *Staphylococcus* spp.		83-86 [3]
aac(6')-Ia, [2]	Aminoglycosides	*Enterobacteriaceae, Pseudomonads*	M18967	
aac(6')-Ic [2]	Aminoglycosides	*Enterobacteriaceae, Pseudomonads*	M94066	
aac(6')-IIa [2]	Aminoglycosides	*Pseudomonads*		112 [4]
aadB [*ant(2")-Ia* [2]]	*Aminoglycosides*	*Enterobacteriaceae*		53-54 [3]
aacC1 [*aac(3)-Ia* [2]]	*Aminoglycosides*	*Pseudomonads*		55-56 [3]
aacC2 [*aac(3)-IIa* [2]]	*Aminoglycosides*	*Pseudomonads*		57-58 [3]
aacC3 [*aac(3)-III* [2]]	*Aminoglycosides*	*Pseudomonads*		59-60 [3]
aacA4 [*aac(6')-Ib* [2]]	*Aminoglycosides*	*Pseudomonads*		65-66 [3]
ant(3")-Ia [2]	Aminoglycosides	*Enterobacteriaceae, Enterococcus* spp., *Staphylococcus* spp.	X02340 M10241	
ant(4')-Ia [2]	Aminoglycosides	*Staphylococcus* spp.	V01282	
aph(3')-Ia [2]	Aminoglycosides	*Enterobacteriaceae, Pseudomonads*	J01839	
aph(3')-IIa [2]	Aminoglycosides	*Enterobacteriaceae, Pseudomonads*	V00618	
aph(3')-IIIa [2]	Aminoglycosides	*Enterococcus* spp., *Staphylococcus* spp.	V01547	
aph(3')-VIa [2]	Aminoglycosides	*Enterobacteriaceae, Pseudomonads*	X07753	
rrs [2]	Streptomycin	*M. tuberculosis*	L15307 S62531	
rpsL [2]	Streptomycin	*M. tuberculosis, M. avium complex*	X80120 U14749 X70995 L08011	
bla_{OXA} [5,6]	ß-lactams	*Enterobacteriaceae, Pseudomonads*		110 [4]
bla_{ROB} [5]	ß-lactams	*Haemophilus* spp. *Pasteurella* spp.		45-48 [3]
bla_{SHV} [5,6]	ß-lactams	*Enterobacteriacea, Pseudomonas aeruginosa*		41-44 [3]
bla_{TEM} [5,6]	ß-lactams	*Enterobacteriaceae, Neisseria* spp., *Haemophilus* spp.		37-40 [3]
bla_{CARB} [5]	ß-lactams	*Pseudomodas* spp., *Enterobacteriaceae*	J05162 S46063 M69058	

Table 5. Antibiotic resistance genes selected for diagnostic purposes (continued)

Genes	Antibiotics	Bacteria[1]	ACCESSION NO.	SEQ ID NO. (gen s)
bla$_{CTX-M-1}$ [5]	ß-lactams	*Enterobacteriaceae*	X92506	
bla$_{CTX-M-2}$ [5]	ß-lactams	*Enterobacteriaceae*	X92507	
bla$_{CMY-2}$ [7]	ß-lactams	*Enterobacteriaceae*	X91840	
bla$_{PER-1}$ [5]	ß-lactams	*Enterobacteriaceae*, *Pseudomodanaceae*	Z21957	
bla$_{PER-2}$ [7]	ß-lactams	*Enterobacteriaceae*	X93314	
bla$_{IMP}$ [5]	ß-lactams	*Enterobacteriaceae*, *Pseudomonas aeruginosa*	AJ223604	
blaZ [12]	ß-lactams	*Enterococcus* spp., *Staphylococcus* spp.		111 [4]
mecA [12]	ß-lactams	*Staphylococcus* spp.		97-98 [3]
penA [13]	ß-lactams	*Neisseria gonorrhoeae*	X54021	
pbp1a [13]	ß-lactams	*Streptococcus pneumoniae*	M90527 X67872 AB006868 AB006874 X67873 AB006878 AB006875 AB006877 AB006879 AF046237 AF046235 AF026431 AF046232 AF046233 AF046236 X67871 Z49095 AF046234 AB006873 X67866 X67868 AB006870 AB006869 AB006872 X67870 AB006871 X67867 X67869 AB006876 AF046230 AF046238 Z49094	
pbp2b [13]	ß-lactams	*Streptococcus pneumoniae*	X16022 M25516 M25518 M25515 U20071 U20084 U20082 U20067 U20079 Z22185 U20072	

Tabl 5. Antibiotic resistance genes s lected for diagn stic purposes (continued)

Genes	Antibiotics	Bacteria[1]	ACCESSION NO.	SEQ ID NO. (genes)
pbp2b [13]	ß-lactams	*Streptococcus pneumoniae*	U20083 U20081 M25522 U20075 U20070 U20077 U20068 Z22184 U20069 U20078 M25521 M25525 M25519 Z21981 M25523 M25526 M25524 Z22230 U20073 U20080 U20074 U20076 M25520 M25517	
pbp2x [13]	ß-lactams	*Streptococcus pneumoniae*	X16367 X65135 AB011204 AB011209 AB011199 AB011200 AB011201 AB011202 AB011198 AB011208 AB011205 AB015852 AB011210 AB015849 AB015850 AB015851 AB015847 AB015846 AB011207 AB015848 Z49096	
int	β -lactams, trimethoprim	*Enterobacteriaceae,*		99-102 [3]
sul	aminoglycosides, antiseptic, chloramphenicol	*Pseudomonads*		103-106 [3]
ermA [14]	Macrolides, lincosamides, streptogramin B	*Staphylococcus* spp.		113 [4]
rmB [14]	Macrolides, lincosamides, streptogramin B	*Enterobacteriaceae, Staphylococcus* spp. *Enterococcus* spp. *Streptococcus* spp.		114 [4]

Table 5. Antibiotic resistance genes selected for diagnostic purposes (continued)

Genes	Antibiotics	Bacteria[1]	ACCESSION NO.	SEQ ID NO. (genes)
ermC [14]	Macrolides, lincosamides, streptogramin B	*Enterobacteriaceae*, *Staphylococcus* spp.		115 [4]
ereA [12]	Macrolides	*Enterobacteriaceae*, *Staphylococcus* spp.	M11277	
ereB [12]	Macrolides	*Enterobacteriaceae* *Staphylococcus* spp.	A15097	
msrA [12]	Macrolides	*Staphylococcus* spp.		77-80 [3]
mtr [8]	Macrolides	*Neisseria gonorrhoeae*	S42418 S40252 S42417 S40251 Z25796 U14993 Q51007 Q51006 Q51073 AF037040 AF037041	
mefA, mefE [8]	Macrolides	*Streptococcus* spp.	U70055 U83667	
mphA [8]	Macrolides	*Enterobacteriaceae*, *Staphylococcus* spp.	D16251	
linA/linA' [9]	Lincosamides	*Staphylococcus* spp.	J03947 M14039	
linB [10]	Lincosamides	*Enterococcus faecium*	AF110130	
rrna [11]	Macrolides	*Mycobacterium avium complex*	U74494	
vga [15]	Streptrogramin	*Staphylococcus* spp.		89-90 [3]
vgb [15]	Streptrogramin	*Staphylococcus* spp.	M36022	
vat [15]	Streptrogramin	*Staphylococcus* spp.		87-88 [3]
vatB [15]	Streptrogramin	*Staphylococcus* spp.	U19456 L38809	
satA [15]	Streptrogramin	*Enterococcus faecium*		81-82 [3]
ileS [12]	Mupirocin	*Staphylococcus aureus*	X74219	
mupA [12]	Mupirocin	*Staphylococcus aureus*	X75439	
gyrA [16]	Quinolones	Gram positive and gram-negative bacteria	X95718 X06744 X57174 X16817 X71437 AF065152 AF060881 D32252	
parC/grlA [16]	Quinolones	Gram-positive and gram-negative bacteria	AB005036 AF056287 X95717 AF129764 AB017811 AF065152	

Table 5. Antibi tic resistance genes selected for diagnostic purposes (continued)

Genes	Antibiotics	Bacteria[1]	ACCESSION NO.	SEQ ID NO. (genes)
parE/grlB [16]	Quinolones	Gram-positive bacteria	X95717 AF065153 AF058920	
norA [16]	Quinolones	*Staphylococcus* spp.	D90119 M80252 M97169	
mexR (nalB) [16]	Quinolones	*Pseudomonas aeruginosa*	U23763	
nfxB [16]	Quinolones	*Pseudomonas aeruginosa*	X65646	
cat [12]	Chloramphenicol	Gram-positive and gram-negative bacteria	M55620 X15100 A24651 M28717 A00568 A00569 X74948 Y00723 A24362 A00569 M93113 M62822 M58516	
rpoB [17]	Rifampin	*Mycobacterium tuberculosis*	AF055891 AF055892 S71246 L27989 AF055893	
inhA [17]	Isoniazid	*Mycobacterium tuberculosis*	AF106077 U02492	
katG [17]	Isoniazid	*Mycobacterium tuberculosis*	U40593 U06259 U06260 U06261 U06262 U40594 U40595	
ahpC [17]	Isoniazid	*Mycobacterium tuberculosis*	U43812 U57761 U24085 U16243 U58030 U18264	
embB [17]	Ethambutol	*Mycobacterium tuberculosis*	U68480	
pncA [17]	Pyrazinamide	*Mycobacterium tuberculosis*	U59967	
vanA [12]	Vancomycin	*Enterococcus* spp.		67-70 [3]
vanB [12]	Vancomycin	*Enterococcus* spp.		116 [4]
vanC1 [12]	Vancomycin	*Enterococcus gallinarum*		117 [4]
vanC2 [12]	Vancomycin	*Enterococcus casseliflavus*	U94521 U94522 U94523 U94524 U94525 L29638	

Tabl 5. Antibi tic resistanc genes selected for diagn stic purp ses (continued)

Genes	Antibiotics	Bacteria[1]	ACCESSION NO.	SEQ ID NO. (genes)
vanC3 [12]	Vancomycin	*Enterococcus flavescens*	L29639 U72706 L29640	
vanD [18]	Vancomycin	*Enterococcus faecium*	AF130997	
tetB [19]	Tetracycline	Gram-negative bacteria	J01830	
tetM [19]	Tetracycline	Gram-negative and gram-positive bacteria	X52632	
sulII [20]	Sulfonamides	Gram-negative bacteria	D37827 M36657 AF017389 AF017391	
dhfrIa [20]	Trimethoprim	Gram-negative bacteria	AJ238350 X17477	
dhfrIb [20]	Trimethoprim	Gram-negative bacteria	Z50805 Z50804	
dhfrV [20]	Trimethoprim	Gram-negative bacteria	X12868	
dhfrVII [20]	Trimethoprim	Gram-negative bacteria	U31119	
dhfrVIII [20]	Trimethoprim	Gram-negative bacteria	U10186	
dhfrIX [20]	Trimethoprim	Gram-negative bacteria	X57730	
dhfrXII [20]	Trimethoprim	Gram-negative bacteria	Z21672	
dfrA [20]	Trimethoprim	*Staphylococcus* spp.	AF045472 U40259 AF051916	

1 Bacteria having high incidence for the specified antibiotic resistance gene. The presence of the antibiotic resistance genes in other bacteria is not excluded.

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3 Antibiotic resistance genes from our co-pending US (N.S. 08/526840) and PCT (PCT/CA/95/00528) patent applications for which we have selected PCR primer pairs.

4 These SEQ ID NO. refer to a previous patent (application WO 98/20157).

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Table 6. List of bacterial toxin selected for diagnostic purposes

Organism	Toxin	Accession number
Actinobacillus actinomycetemcomitans	Cytolethal distending toxin (*cdt*A, *cdt*B, *cdt*C)	AF006830
	Leukotoxin (*ltx*A)	M27399
Actinomyces pyogenes	Hemolysin (pyolysin)	U84782
Aeromonas hydrophila	Aerolysin	M16495
		U81555
Bacillus anthracis	Anthrax toxin (*cya*)	M23179
Bacillus cereus	Enterotoxin (*bce*T)	D17312
Bacteroides fragilis	Metalloprotease toxin-3	AF056297
	Metalloprotease toxin-2	U90931
		AF081785
Bordetella bronchiseptica	Adenylate cyclase hemolysin (*cya*A)	Z37112, U22953
Dermonecrotic toxin (*dnt*)		U59687
		AB020025
Bordetella pertussis	Pertussis toxin (S1 subunit, *tox*)	AJ006151
		AJ006153
	Patent:	AJ006155
	EP0322533-A 2 05jul89	AJ006157
	EP0322115-A 5 28jun89	AJ006159
	EP0396964-A 1 14nov90	AJ007363
	JP1987228286-A 1 7oct87	M14378, M16494
		AJ007364
		M13223
		X16347
	Adenyl cyclase (*cya*)	18323
	Dermonecrotic toxin (*dnt*)	U10527
Campylobacter jejuni	Cytolethal distending toxin (*cdt*A, *cdt*B, *cdt*C)	U51121
Citrobacter freundii	Shiga-like toxin (*slt*-IIcA)	X67514, S53206
Clostridium botulinum	Botulism toxin (BoNT)	X52066, X52088
		X73423
	The A,B,E and F serotypes are	M30196
	neurotoxic for human	X70814
		X70819
	The other serotypes have not be considered	X71343
		Z11934
	Partial sequences (<200 bp) have	X70817
	not be considered	M81186
		X70818
		X70815
		X62089
		X62683
		S76749
		X81714
		X70816
		X70820
		X70281
		L35496
		M92906

Table 6. List of bacterial toxins selected for diagnostic purposes (continued)

Organism	Toxin	Accession number
Clostridium difficile	A toxin (enterotoxin) (*tcd*A)	AB012304 AF053400 Y12616 X51797 X17194 M30307
	B (cytotoxin) (*tox*B)	Z23277 X53138
Clostridium perfringens	Alpha (phospholipase C) (*cpa*)	L43545 L43546 L43547 L43548 X13608 X17300 D10248
	Beta (dermonecrotic protein) (*cpb*)	L13198 X83275 L77965
	Enterotoxin (*cpe*) Pseudogene (not expressed)	AF037328 AF037329 AF037330
	Epsilon toxin (*etx*D)	M80837 M95206 X60694
	Iota (Ia et Ib) Lambda (metalloprotease) Theta (perfringolysin O)	X73562 D45904 M36704
Clostridium tetani	Tetanos toxin	X06214 X04436
Corynebacterium diphtheriae	Diphtheriae toxin Patent: JP 1985227681-A/1	X00703
Corynebacterium pseudotuberculosis	Phospholipase C Patent: WO 9011351-A 2	A21336
Enterobacter cloacae	Shiga-like toxin II	Z50754, U33502
Escherichia coli (EHEC)	Hemolysin toxin (*hly*A and *ehx*A)	AF043471 X94129 X79839 X86087 AB011549 AF074613
	Shiga-like (Vero cytotoxin) (*stx*) Contain the sequences for both the A and B subunits Patent:JP 1995008280-A/1	X81418 M14107 M10133 M12863 X81417 X81416 X81415 Z36900 L11078 L04539 L11079 X65949 M21534 M29153 Z37725 Z36901 X61283 AB017524 U72191

Table 6. List of bacterial toxins selected for diagnostic purposes (continued)

Organism	Toxin	Accession number
Escherichia coli (ETEC)	Enterotoxin (heat-labile) (*elt*B) Patent: EP 0145486-A 5 WO 9313202-A 42 JP 1986005097-A JP 1992320675-A	M17874 M17873 J01605
	Enterotoxin (heat-stable) (*ast*A) (*est*A1)	L11241 M58746 M29255 V00612 J01831
Escherichia coli(other)	*Cytolethal-distending toxin* (*cdt*) (3 genes)	U03293 U04208 U89305
	Cytotoxic necrotizing factor 1 (*cnf*1)	U42629
	Microcin 24 (*mtf*S)	U47048
	Autotransporter enterotoxin (*Pet*) (cytotoxin)	AF056581
Haemophilus ducreyi	Cytolethal distending toxin (*cdt*A, *cdt*B, *cdt*C)	U53215
Helicobacter pylori	Vacuolating toxin (*vac*A)	U07145 U80067 U80068 AF077938 AF077939 AF077940 AF077941
Pasteurella multocida	Mitogenic toxin (dermonecrotic toxin)	X57775, Z28388 X51512 X52478
Pseudomonas aeruginosa	Cytotoxin (Enterotoxin A)	X14956
Shigella dysenteriae type 1	Shiga toxin (2 subunits) (*stx*A et *stx*B)	X07903, M32511 M19437 M24352, M21947
Staphylococcus aureus	Gamma-hemolysin (*hlg*2)	D42143 L01055
	Enterotoxin	U93688
	Enterotoxin C1 (*ent*C1)	X05815
	Enterotoxin H (*seh*)	U11702
	Exfoliative toxin A (ETA)	M17347
	(Epidermolytic toxin A)	M17357 L25372, M20371
	Exfoliative toxin B (ETB)	M17348, M13775
	Leukocidin R (F and S component, *luk*F and *luk*S)	X64389, S53213
	(Hemolysin B and C)	X72700 L01055
	Toxic shock syndrome toxin 1 (TSST-1)	X01645
	(alpha toxin)	M90536
	(alpha hemolysin)	J02615 U93688
Staphylococcus epidermidis	Delta toxin (*hld*)	AF068634
Staphylococcus intermedius	Leukocidin R (F and S component, *luk*F and *luk*S) (synergohymenotropic toxin)	X79188
Streptococcus pneumoniae	Pneumolysin	X52474
Streptococcus pyogenes	Streptococcal pyrogenic exotoxin A (*spe*A)	X61560 (et 19 autres) X03929 U40453, M19350
	Pyrogenic exotoxin B (*spe*B)	U63134 M86905, M35110

Table 6. List of bacterial toxin select d f r diagnostic purposes (continued)

Organism	Toxin Accession number	
Vibrio cholerae	Cholerae toxin (*ctx*A et *ctx*B subunits)	X00171
	Patent: X76390	
	JP 1995008279-A 1	X58786
	EP 0368819-A 12 (*ctx*B)	X58785, S55782
	WO 9313202-A 45 (*ctx*A)	D30052
		D30053
		K02679
	Accessory cholera enterotoxin (*ace*)	Z22569
	Zonula occludens toxin, (*zot*)	M83563
Vibrio parahaemolyticus	Thermostable direct hemolysin (*tdh*)	S67841
Vibrio vulnificus	Cytolysin (*vvh*A)	M34670
Yersinia enterocolitica	Heat-stable enterotoxin (*yst*C)	D63578
Yersinia pestis	Toxin	X92727

Table 7. Origin of the sequences in the sequenc listing.

SEQ ID NO.	Bacterial,fungal or parasitical species	Source	Comments*
1	*Acinetobacter baumannii*	This patent	*tuf*
2	*Actinomyces meyeri*	This patent	*tuf*
3	*Aerococcus viridans*	This patent	*tuf*
4	*Achromobacter xylosoxidans* subsp. *denitrificans*	This patent	*tuf*
5	*Anaerorhabdus furcosus*	This patent	*tuf*
6	*Bacillus anthracis*	This patent	*tuf*
7	*Bacillus cereus*	This patent	*tuf*
8	*Bacteroides distasonis*	This patent	*tuf*
9	*Enterococcus casseliflavus*	This patent	*tuf*
10	*Staphylococcus saprophyticus*	This patent	*tuf*
11	*Bacteroides vulgatus*	This patent	*tuf*
12	*Bartonella henselae*	This patent	*tuf*
13	*Bifidobacterium adolescentis*	This patent	*tuf*
14	*Bifidobacterium dentium*	This patent	*tuf*
15	*Brucella abortus*	This patent	*tuf*
16	*Burkholderia cepacia*	This patent	*tuf*
17	*Cedecea davisae*	This patent	*tuf*
18	*Cedecea neteri*	This patent	*tuf*
19	*Cedecea lapagei*	This patent	*tuf*
20	*Chlamydia pneumoniae*	This patent	*tuf*
21	*Chlamydia psittaci*	This patent	*tuf*
22	*Chlamydia trachomatis*	This patent	*tuf*
23	*Chryseobacterium meningosepticum*	This patent	*tuf*
24	*Citrobacter amalonaticus*	This patent	*tuf*
25	*Citrobacter braakii*	This patent	*tuf*
26	*Citrobacter koseri*	This patent	*tuf*
27	*Citrobacter farmeri*	This patent	*tuf*
28	*Citrobacter freundii*	This patent	*tuf*
29	*Citrobacter sedlakii*	This patent	*tuf*
30	*Citrobacter werkmanii*	This patent	*tuf*
31	*Citrobacter youngae*	This patent	*tuf*
32	*Clostridium perfringens*	This patent	*tuf*
33	*Comamonas acidovorans*	This patent	*tuf*
34	*Corynebacterium bovis*	This patent	*tuf*
35	*Corynebacterium cervicis*	This patent	*tuf*
36	*Corynebacterium flavescens*	This patent	*tuf*
37	*Corynebacterium kutscheri*	This patent	*tuf*
38	*Corynebacterium minutissimum*	This patent	*tuf*
39	*Corynebacterium mycetoides*	This patent	*tuf*
40	*Corynebacterium pseudogenitalium*	This patent	*tuf*
41	*Corynebacterium renale*	This patent	*tuf*
42	*Corynebacterium ulcerans*	This patent	*tuf*
43	*Corynebacterium urealyticum*	This patent	*tuf*
44	*Corynebacterium xerosis*	This patent	*tuf*
45	*Coxiella burnetii*	This patent	*tuf*
46	*Edwardsiella hoshinae*	This patent	*tuf*
47	*Edwardsiella tarda*	This patent	*tuf*
48	*Eikenella corrodens*	This patent	*tuf*
49	*Enterobacter aerogenes*	This patent	*tuf*
50	*Enterobacter agglomerans*	This patent	*tuf*
51	*Enterobacter amnigenus*	This patent	*tuf*
52	*Enterobacter asburiae*	This patent	*tuf*
53	*Enterobacter cancerogenus*	This patent	*tuf*
54	*Enterobacter cloacae*	This patent	*tuf*
55	*Enterobacter gergoviae*	This patent	*tuf*
56	*Enterobacter hormaechei*	This patent	*tuf*
57	*Enterobacter sakazakii*	This patent	*tuf*
58	*Enterococcus casseliflavus*	This patent	*tuf*
59	*Enterococcus cecorum*	This patent	*tuf*
60	*Enterococcus dispar*	This patent	*tuf*
61	*Enterococcus durans*	This patent	*tuf*

Table 7. Origin of the sequences in the sequence listing. (continued)

SEQ ID NO.	Bacterial,fungal or parasitical species	Source	Comments*
62	*Enterococcus faecalis*	This patent	*tuf*
63	*Ent rococcus faecalis*	This patent	*tuf*
64	*Enterococcus faecium*	This patent	*tuf*
65	*Enterococcus flavescens*	This patent	*tuf*
66	*Enterococcus gallinarum*	This patent	*tuf*
67	*Enterococcus hirae*	This patent	*tuf*
68	*Enterococcus mundtii*	This patent	*tuf*
69	*Enterococcus pseudoavium*	This patent	*tuf*
70	*Enterococcus raffinosus*	This patent	*tuf*
71	*Enterococcus saccharolyticus*	This patent	*tuf*
72	*Enterococcus solitarius*	This patent	*tuf*
73	*Enterococcus casseliflavus*	This patent	*tuf* (C)
74	*Enterococcus faecium*	This patent	*tuf* (C)
75	*Enterococcus flavescens*	This patent	*tuf* (C)
76	*Enterococcus gallinarum*	This patent	*tuf* (C)
77	*Ehrlichia canis*	This patent	*tuf*
78	*Escherichia coli*	This patent	*tuf*
79	*Escherichia fergusonii*	This patent	*tuf*
80	*Escherichia hermannii*	This patent	*tuf*
81	*Escherichia vulneris*	This patent	*tuf*
82	*Eubacterium lentum*	This patent	*tuf*
83	*Eubacterium nodatum*	This patent	*tuf*
84	*Ewingella americana*	This patent	*tuf*
85	*Francisella tularensis*	This patent	*tuf*
86	*Fusobacterium nucleatum* subsp. *polymorphum*	This patent	*tuf*
87	*Gemella haemolysans*	This patent	*tuf*
88	*Gemella morbillorum*	This patent	*tuf*
89	*Haemophilus actinomycetemcomitans*	This patent	*tuf*
90	*Haemophilus aphrophilus*	This patent	*tuf*
91	*Haemophilus ducreyi*	This patent	*tuf*
92	*Haemophilus haemolyticus*	This patent	*tuf*
93	*Haemophilus parahaemolyticus*	This patent	*tuf*
94	*Haemophilus parainfluenzae*	This patent	*tuf*
95	*Haemophilus paraphrophilus*	This patent	*tuf*
96	*Haemophilus segnis*	This patent	*tuf*
97	*Hafnia alvei*	This patent	*tuf*
98	*Kingella kingae*	This patent	*tuf*
99	*Klebsiella omithinolytica*	This patent	*tuf*
100	*Klebsiella oxytoca*	This patent	*tuf*
101	*Klebsiella planticola*	This patent	*tuf*
102	*Klebsiella pneumoniae* subsp. *ozaenae*	This patent	*tuf*
103	*Klebsiella pneumoniae* subsp. *pneumoniae*	This patent	*tuf*
104	*Klebsiella pneumoniae* subsp. *rhinoscleromatis*	This patent	*tuf*
105	*Kluyvera ascorbata*	This patent	*tuf*
106	*Kluyvera cryocrescens*	This patent	*tuf*
107	*Kluyvera georgiana*	This patent	*tuf*
108	*Lactobacillus casei* subsp. *casei*	This patent	*tuf*
109	*Lactococcus lactis* subsp. *lactis*	This patent	*tuf*
110	*Leclercia adecarboxylata*	This patent	*tuf*
111	*Legionella micdadei*	This patent	*tuf*
112	*Legionella pneumophila* subsp. *pneumophila*	This patent	*tuf*
113	*Leminorella grimontii*	This patent	*tuf*
114	*Leminorella richardii*	This patent	*tuf*
115	*Leptospira interrogans*	This patent	*tuf*
116	*Megamonas hypermegale*	This patent	*tuf*
117	*Mitsuokella multacida*	This patent	*tuf*
118	*Mobiluncus curtisii* subsp. *holmesii*	This patent	*tuf*
119	*Moellerella wisconsensis*	This patent	*tuf*
120	*Branhamella catarrhalis*	This patent	*tuf*
121	*Morganella morganii* subsp. *morganii*	This patent	*tuf*
122	*Mycobacterium tuberculosis*	This patent	*tuf*

Table 7. Origin f the sequences in the sequence listing. (continued)

SEQ ID NO.	Bacterial,fungal or parasitical species	Source	Comments*
123	*Neisseria cinerea*	This patent	*tuf*
124	*Neisseria elongata* subsp. *elongata*	This patent	*tuf*
125	*Neiss ria flavescens*	This patent	*tuf*
126	*Neisseria gonorrhoeae*	This patent	*tuf*
127	*Neisseria lactamica*	This patent	*tuf*
128	*Neisseria meningitidis*	This patent	*tuf*
129	*Neisseria mucosa*	This patent	*tuf*
130	*Neisseria sicca*	This patent	*tuf*
131	*Neisseria subflava*	This patent	*tuf*
132	*Neisseria weaveri*	This patent	*tuf*
133	*Ochrobactrum anthropi*	This patent	*tuf*
134	*Pantoea agglomerans*	This patent	*tuf*
135	*Pantoea dispersa*	This patent	*tuf*
136	*Pasteurella multocida*	This patent	*tuf*
137	*Peptostreptococcus anaerobius*	This patent	*tuf*
138	*Peptostreptococcus asaccharolyticus*	This patent	*tuf*
139	*Peptostreptococcus prevotii*	This patent	*tuf*
140	*Porphyromonas asaccharolytica*	This patent	*tuf*
141	*Porphyromonas gingivalis*	This patent	*tuf*
142	*Pragia fontium*	This patent	*tuf*
143	*Prevotella melaninogenica*	This patent	*tuf*
144	*Prevotella oralis*	This patent	*tuf*
145	*Propionibacterium acnes*	This patent	*tuf*
146	*Proteus mirabilis*	This patent	*tuf*
147	*Proteus penneri*	This patent	*tuf*
148	*Proteus vulgaris*	This patent	*tuf*
149	*Providencia alcalifaciens*	This patent	*tuf*
150	*Providencia rettgeri*	This patent	*tuf*
151	*Providencia rustigianii*	This patent	*tuf*
152	*Providencia stuartii*	This patent	*tuf*
153	*Pseudomonas aeruginosa*	This patent	*tuf*
154	*Pseudomonas fluorescens*	This patent	*tuf*
155	*Pseudomonas stutzeri*	This patent	*tuf*
156	*Psychrobacter phenylpyruvicus*	This patent	*tuf*
157	*Rahnella aquatilis*	This patent	*tuf*
158	*Salmonella choleraesuis* subsp.*arizonae*	This patent	*tuf*
159	*Salmonella choleraesuis* subsp. *choleraesuis* serotype *choleraesuis*	This patent	*tuf*
160	*Salmonella choleraesuis* subsp. *diarizonae*	This patent	*tuf*
161	*Salmonella choleraesuis* subsp. *choleraesuis* serotype *heidelberg*	This patent	*tuf*
162	*Salmonella choleraesuis* subsp. *houtenae*	This patent	*tuf*
163	*Salmonella choleraesuis* subsp. *indica*	This patent	*tuf*
164	*Salmonella choleraesuis* subsp. *salamae*	This patent	*tuf*
165	*Salmonella choleraesuis* subsp. *choleraesuis* serotype *typhi*	This patent	*tuf*
166	*Serratia fonticola*	This patent	*tuf*
167	*Serratia liquefaciens*	This patent	*tuf*
168	*Serratia marcescens*	This patent	*tuf*
169	*Serratia odorifera*	This patent	*tuf*
170	*Serratia plymuthica*	This patent	*tuf*
171	*Serratia rubidaea*	This patent	*tuf*
172	*Shigella boydii*	This patent	*tuf*
173	*Shigella dysenteriae*	This patent	*tuf*
174	*Shigella flexneri*	This patent	*tuf*
175	*Shigella sonnei*	This patent	*tuf*
176	*Staphylococcus aureus*	This patent	*tuf*
177	*Staphylococcus aureus*	This patent	*tuf*
178	*Staphylococcus aureus*	This patent	*tuf*
179	*Staphylococcus aureus*	This patent	*tuf*
180	*Staphylococcus aureus* subsp. *aureus*	This patent	*tuf*
181	*Staphylococcus auricularis*	This patent	*tuf*
182	*Staphylococcus capitis* subsp. *capitis*	This patent	*tuf*
183	*Staphylococcus caseolyticus*	This patent	*tuf*

Table 7. Origin of the sequences in the sequence listing. (continued)

SEQ ID NO.	Bacterial,fungal or parasitical species	Source	Comments*
184	*Staphylococcus cohnii*	This pat nt	*tuf*
185	*Staphylococcus epidermidis*	This patent	*tuf*
186	*Staphylococcus haemolyticus*	This patent	*tuf*
187	*Staphylococcus warneri*	This patent	*tuf*
188	*Staphylococcus haemolyticus*	This patent	*tuf*
189	*Staphylococcus haemolyticus*	This patent	*tuf*
190	*Staphylococcus haemolyticus*	This patent	*tuf*
191	*Staphylococcus hominis* subsp. *hominis*	This patent	*tuf*
192	*Staphylococcus warneri*	This patent	*tuf*
193	*Staphylococcus hominis*	This patent	*tuf*
194	*Staphylococcus hominis*	This patent	*tuf*
195	*Staphylococcus hominis*	This patent	*tuf*
196	*Staphylococcus hominis*	This patent	*tuf*
197	*Staphylococcus lugdunensis*	This patent	*tuf*
198	*Staphylococcus saprophyticus*	This patent	*tuf*
199	*Staphylococcus saprophyticus*	This patent	*tuf*
200	*Staphylococcus saprophyticus*	This patent	*tuf*
201	*Staphylococcus sciuri* subsp. *sciuri*	This patent	*tuf*
202	*Staphylococcus warneri*	This patent	*tuf*
203	*Staphylococcus warneri*	This patent	*tuf*
204	*Bifidobacterium longum*	This patent	*tuf*
205	*Stenotrophomonas maltophilia*	This patent	*tuf*
206	*Streptococcus acidominimus*	This patent	*tuf*
207	*Streptococcus agalactiae*	This patent	*tuf*
208	*Streptococcus agalactiae*	This patent	*tuf*
209	*Streptococcus agalactiae*	This patent	*tuf*
210	*Streptococcus agalactiae*	This patent	*tuf*
211	*Streptococcus anginosus*	This patent	*tuf*
212	*Streptococcus bovis*	This patent	*tuf*
213	*Streptococcus anginosus*	This patent	*tuf*
214	*Streptococcus cricetus*	This patent	*tuf*
215	*Streptococcus cristatus*	This patent	*tuf*
216	*Streptococcus downei*	This patent	*tuf*
217	*Streptococcus dysgalactiae*	This patent	*tuf*
218	*Streptococcus equi* subsp. *equi*	This patent	*tuf*
219	*Streptococcus ferus*	This patent	*tuf*
220	*Streptococcus gordonii*	This patent	*tuf*
221	*Streptococcus anginosus*	This patent	*tuf*
222	*Streptococcus macacae*	This patent	*tuf*
223	*Streptococcus gordonii*	This patent	*tuf*
224	*Streptococcus mutans*	This patent	*tuf*
225	*Streptococcus parasanguinis*	This patent	*tuf*
226	*Streptococcus ratti*	This patent	*tuf*
227	*Streptococcus sanguinis*	This patent	*tuf*
228	*Streptococcus sobrinus*	This patent	*tuf*
229	*Streptococcus suis*	This patent	*tuf*
230	*Streptococcus uberis*	This patent	*tuf*
231	*Streptococcus vestibularis*	This patent	*tuf*
232	*Tatumella ptyseos*	This patent	*tuf*
233	*Trabulsiella guamensis*	This patent	*tuf*
234	*Veillonella parvula*	This patent	*tuf*
235	*Yersinia enterocolitica*	This patent	*tuf*
236	*Yersinia frederiksenii*	This patent	*tuf*
237	*Yersinia intermedia*	This patent	*tuf*
238	*Yersinia pestis*	This patent	*tuf*
239	*Yersinia pseudotuberculosis*	This patent	*tuf*
240	*Yersinia rohdei*	This patent	*tuf*
241	*Yokenella regensburgei*	This patent	*tuf*
242	*Achromobacter xylosoxidans* subsp. *denitrificans*	This patent	*atpD*
243	*Acinetobacter baumannii*	This patent	*atpD*
244	*Acinetobacter lwoffii*	This patent	*atpD*

Table 7. Origin f the sequenc s in the sequence listing. (c ntinued)

SEQ ID NO.	Bacterial,fungal or parasitical species	Source	Comments*
245	*Staphylococcus saprophyticus*	This patent	*atpD*
246	*Alcaligenes faecalis*	This patent	*atpD*
247	*Bacillus anthracis*	This patent	*atpD*
248	*Bacillus cereus*	This patent	*atpD*
249	*Bacteroides distasonis*	This patent	*atpD*
250	*Bacteroides ovatus*	This patent	*atpD*
251	*Leclercia adecarboxylata*	This patent	*atpD*
252	*Stenotrophomonas maltophilia*	This patent	*atpD*
253	*Bartonella henselae*	This patent	*atpD*
254	*Bifidobacterium adolescentis*	This patent	*atpD*
255	*Brucella abortus*	This patent	*atpD*
256	*Cedecea davisae*	This patent	*atpD*
257	*Cedecea lapagei*	This patent	*atpD*
258	*Cedecea neteri*	This patent	*atpD*
259	*Chryseobacterium meningosepticum*	This patent	*atpD*
260	*Citrobacter amalonaticus*	This patent	*atpD*
261	*Citrobacter braakii*	This patent	*atpD*
262	*Citrobacter koseri*	This patent	*atpD*
263	*Citrobacter farmeri*	This patent	*atpD*
264	*Citrobacter freundii*	This patent	*atpD*
265	*Citrobacter koseri*	This patent	*atpD*
266	*Citrobacter sedlakii*	This patent	*atpD*
267	*Citrobacter werkmanii*	This patent	*atpD*
268	*Citrobacter youngae*	This patent	*atpD*
269	*Clostridium innocuum*	This patent	*atpD*
270	*Clostridium perfringens*	This patent	*atpD*
272	*Corynebacterium diphtheriae*	This patent	*atpD*
273	*Corynebacterium pseudodiphtheriticum*	This patent	*atpD*
274	*Corynebacterium ulcerans*	This patent	*atpD*
275	*Corynebacterium urealyticum*	This patent	*atpD*
276	*Coxiella burnetii*	This patent	*atpD*
277	*Edwardsiella hoshinae*	This patent	*atpD*
278	*Edwardsiella tarda*	This patent	*atpD*
279	*Eikenella corrodens*	This patent	*atpD*
280	*Enterobacter agglomerans*	This patent	*atpD*
281	*Enterobacter amnigenus*	This patent	*atpD*
282	*Enterobacter asburiae*	This patent	*atpD*
283	*Enterobacter cancerogenus*	This patent	*atpD*
284	*Enterobacter cloacae*	This patent	*atpD*
285	*Enterobacter gergoviae*	This patent	*atpD*
286	*Enterobacter hormaechei*	This patent	*atpD*
287	*Enterobacter sakasakii*	This patent	*atpD*
288	*Enterococcus avium*	This patent	*atpD*
289	*Enterococcus casseliflavus*	This patent	*atpD*
290	*Enterococcus durans*	This patent	*atpD*
291	*Enterococcus faecalis*	This patent	*atpD*
292	*Enterococcus faecium*	This patent	*atpD*
293	*Enterococcus gallinarum*	This patent	*atpD*
294	*Enterococcus saccharolyticus*	This patent	*atpD*
295	*Escherichia fergusonii*	This patent	*atpD*
296	*Escherichia hermannii*	This patent	*atpD*
297	*Escherichia vulneris*	This patent	*atpD*
298	*Eubacterium lentum*	This patent	*atpD*
299	*Ewingella americana*	This patent	*atpD*
300	*Francisella tularensis*	This patent	*atpD*
301	*Fusobacterium gonidiaformans*	This patent	*atpD*
302	*Fusobacterium necrophorum* subsp. *necrophorum*	This patent	*atpD*
303	*Fusobacterium nucleatum* subsp. *polymorphum*	This patent	*atpD*
304	*Gardnerella vaginalis*	This patent	*atpD*
305	*Gemella haemolysans*	This patent	*atpD*
306	*Gemella morbillorum*	This patent	*atpD*

Tabl 7. Origin of the sequences in the sequence listing. (c ntinued)

SEQ ID NO.	Bacterial,fungal or parasitical species	Source	Comments*
307	*Haemophilus ducreyi*	This patent	*atpD*
308	*Haemophilus haemolyticus*	This patent	*atpD*
309	*Haemophilus parahaemolyticus*	This patent	*atpD*
310	*Haemophilus parainfluenzae*	This patent	*atpD*
311	*Hafnia alvei*	This patent	*atpD*
312	*Kingella kingae*	This patent	*atpD*
313	*Klebsiella pneumoniae* subsp. *ozaenae*	This patent	*atpD*
314	*Klebsiella ornithinolytica*	This patent	*atpD*
315	*Klebsiella oxytoca*	This patent	*atpD*
316	*Klebsiella planticola*	This patent	*atpD*
317	*Klebsiella pneumoniae* subsp. *pneumoniae*	This patent	*atpD*
318	*Kluyvera ascorbata*	This patent	*atpD*
319	*Kluyvera cryocrescens*	This patent	*atpD*
320	*Kluyvera georgiana*	This patent	*atpD*
321	*Lactobacillus acidophilus*	This patent	*atpD*
322	*Legionella pneumophila* subsp. *pneumophila*	This patent	*atpD*
323	*Leminorella grimontii*	This patent	*atpD*
324	*Listeria monocytogenes*	This patent	*atpD*
325	*Micrococcus lylae*	This patent	*atpD*
326	*Moellerella wisconsensis*	This patent	*atpD*
327	*Branhamella catarrhalis*	This patent	*atpD*
328	*Moraxella osloensis*	This patent	*atpD*
329	*Morganella morganii* subsp. *morganii*	This patent	*atpD*
330	*Pantoea agglomerans*	This patent	*atpD*
331	*Pantoea dispersa*	This patent	*atpD*
332	*Pasteurella multocida*	This patent	*atpD*
333	*Pragia fontium*	This patent	*atpD*
334	*Proteus mirabilis*	This patent	*atpD*
335	*Proteus vulgaris*	This patent	*atpD*
336	*Providencia alcalifaciens*	This patent	*atpD*
337	*Providencia rettgeri*	This patent	*atpD*
338	*Providencia rustigianii*	This patent	*atpD*
339	*Providencia stuartii*	This patent	*atpD*
340	*Psychrobacter phenylpyruvicus*	This patent	*atpD*
341	*Rahnella aquatilis*	This patent	*atpD*
342	*Salmonella choleraesuis* subsp. *arizonae*	This patent	*atpD*
343	*Salmonella choleraesuis* subsp. *choleraesuis* serotype *choleraesuis*	This patent	*atpD*
344	*Salmonella choleraesuis* subsp. *diarizonae*	This patent	*atpD*
345	*Salmonella choleraesuis* subsp. *houtenae*	This patent	*atpD*
346	*Salmonella choleraesuis* subsp. *indica*	This patent	*atpD*
347	*Salmonella choleraesuis* subsp. *choleraesuis* serotype *paratyphi* A	This patent	*atpD*
348	*Salmonella choleraesuis* subsp. *choleraesuis* serotype *paratyphi* B	This patent	*atpD*
349	*Salmonella choleraesuis* subsp. *salamae*	This patent	*atpD*
350	*Salmonella choleraesuis* subsp. *choleraesuis* serotype *typhi*	This patent	*atpD*
351	*Salmonella choleraesuis* subsp. *choleraesuis* serotype *typhimurium*	This patent	*atpD*
352	*Salmonella choleraesuis* subsp. *choleraesuis* serotype *virchow*	This patent	*atpD*
353	*Serratia ficaria*	This patent	*atpD*
354	*Serratia fonticola*	This patent	*atpD*
355	*Serratia grimesii*	This patent	*atpD*
356	*Serratia liquefaciens*	This patent	*atpD*
357	*Serratia marcescens*	This patent	*atpD*
358	*Serratia odorifera*	This patent	*atpD*
359	*Serratia plymuthica*	This patent	*atpD*
360	*Serratia rubidaea*	This patent	*atpD*
361	*Shewanella putida*	This patent	*atpD*
362	*Shigella boydii*	This patent	*atpD*
363	*Shigella dysenteriae*	This patent	*atpD*
364	*Shigella flexneri*	This patent	*atpD*
365	*Shigella sonnei*	This patent	*atpD*
366	*Staphylococcus aureus*	This patent	*atpD*
367	*Staphylococcus auricularis*	This patent	*atpD*

Table 7. Origin f the sequences in the sequence listing. (continued)

SEQ ID NO.	Bacterial,fungal or parasitical species	Source	Comments*
368	*Staphylococcus capitis* subsp. *capitis*	This patent	*atpD*
369	*Staphylococcus cohnii*	This patent	*atpD*
370	*Staphylococcus epidermidis*	This patent	*atpD*
371	*Staphylococcus haemolyticus*	This patent	*atpD*
372	*Staphylococcus hominis* subsp. *hominis*	This patent	*atpD*
373	*Staphylococcus hominis*	This patent	*atpD*
374	*Staphylococcus lugdunensis*	This patent	*atpD*
375	*Staphylococcus saprophyticus*	This patent	*atpD*
376	*Staphylococcus simulans*	This patent	*atpD*
377	*Staphylococcus warneri*	This patent	*atpD*
378	*Streptococcus acidominimus*	This patent	*atpD*
379	*Streptococcus agalactiae*	This patent	*atpD*
380	*Streptococcus agalactiae*	This patent	*atpD*
381	*Streptococcus agalactiae*	This patent	*atpD*
382	*Streptococcus agalactiae*	This patent	*atpD*
383	*Streptococcus agalactiae*	This patent	*atpD*
384	*Streptococcus dysgalactiae*	This patent	*atpD*
385	*Streptococcus equi* subsp. *equi*	This patent	*atpD*
386	*Streptococcus anginosus*	This patent	*atpD*
387	*Streptococcus salivarius*	This patent	*atpD*
388	*Streptococcus suis*	This patent	*atpD*
389	*Streptococcus uberis*	This patent	*atpD*
390	*Tatumella ptyseos*	This patent	*atpD*
391	*Trabulsiella guamensis*	This patent	*atpD*
392	*Yersinia bercovieri*	This patent	*atpD*
393	*Yersinia enterocolitica*	This patent	*atpD*
394	*Yersinia frederiksenii*	This patent	*atpD*
395	*Yersinia intermedia*	This patent	*atpD*
396	*Yersinia pseudotuberculosis*	This patent	*atpD*
397	*Yersinia rohdei*	This patent	*atpD*
398	*Yokenella regensburgei*	This patent	*atpD*
399	*Yarrowia lipolytica*	This patent	*tuf* (ef-1)
400	*Absidia corymbifera*	This patent	*tuf* (ef-1)
401	*Alternaria alternata*	This patent	*tuf* (ef-1)
402	*Aspergillus flavus*	This patent	*tuf* (ef-1)
403	*Aspergillus fumigatus*	This patent	*tuf* (ef-1)
404	*Aspergillus fumigatus*	This patent	*tuf* (ef-1)
405	*Aspergillus niger*	This patent	*tuf* (ef-1)
406	*Blastoschizomyces capitatus*	This patent	*tuf* (ef-1)
407	*Candida albicans*	This patent	*tuf* (ef-1)
408	*Candida albicans*	This patent	*tuf* (ef-1)
409	*Candida albicans*	This patent	*tuf* (ef-1)
410	*Candida albicans*	This patent	*tuf* (ef-1)
411	*Candida albicans*	This patent	*tuf* (ef-1)
412	*Candida dubliniensis*	This patent	*tuf* (ef-1)
413	*Candida catenulata*	This patent	*tuf* (ef-1)
414	*Candida dubliniensis*	This patent	*tuf* (ef-1)
415	*Candida dubliniensis*	This patent	*tuf* (ef-1)
416	*Candida famata*	This patent	*tuf* (ef-1)
417	*Candida glabrata*	This patent	*tuf* (ef-1)
418	*Candida guilliermondii*	This patent	*tuf* (ef-1)
419	*Candida haemulonii*	This patent	*tuf* (ef-1)
420	*Candida inconspicua*	This patent	*tuf* (ef-1)
421	*Candida kefyr*	This patent	*tuf* (ef-1)
422	*Candida krusei*	This patent	*tuf* (ef-1)
423	*Candida lambica*	This patent	*tuf* (ef-1)
424	*Candida lusitaniae*	This patent	*tuf* (ef-1)
425	*Candida norvegensis*	This patent	*tuf* (ef-1)
426	*Candida parapsilosis*	This patent	*tuf* (ef-1)
427	*Candida rugosa*	This patent	*tuf* (ef-1)
428	*Candida sphaerica*	This patent	*tuf* (ef-1)

Table 7. **Origin of the sequences in the sequence listing. (continued)**

SEQ ID NO.	Bacterial,fungal or parasitical species	Source	Comments*
429	*Candida tropicalis*	This patent	*tuf* (ef-1)
430	*Candida utilis*	This patent	*tuf* (ef-1)
431	*Candida viswanathii*	This patent	*tuf* (ef-1)
432	*Candida zeylanoides*	This patent	*tuf* (ef-1)
433	*Coccidioides immitis*	This patent	*tuf* (ef-1)
434	*Cryptococcus albidus*	This patent	*tuf* (ef-1)
435	*Exophiala jeanselmei*	This patent	*tuf* (ef-1)
436	*Fusarium oxysporum*	This patent	*tuf* (ef-1)
437	*Geotrichum* spp.	This patent	*tuf* (ef-1)
438	*Histoplasma capsulatum*	This patent	*tuf* (ef-1)
439	*Issatchenkia orientalis kudrjanzev*	This patent	*tuf* (ef-1)
440	*Malassezia furfur*	This patent	*tuf* (ef-1)
441	*Malassezia pachydermatis*	This patent	*tuf* (ef-1)
442	*Malbranchea filamentosa*	This patent	*tuf* (ef-1)
443	*Metschnikowia pulcherrima*	This patent	*tuf* (ef-1)
444	*Paecilomyces lilacinus*	This patent	*tuf* (ef-1)
445	*Paracoccidioides brasiliensis*	This patent	*tuf* (ef-1)
446	*Penicillium marneffei*	This patent	*tuf* (ef-1)
447	*Pichia anomala*	This patent	*tuf* (ef-1)
448	*Pichia anomala*	This patent	*tuf* (ef-1)
449	*Pseudallescheria boydii*	This patent	*tuf* (ef-1)
450	*Rhizopus oryzae*	This patent	*tuf* (ef-1)
451	*Rhodotorula minuta*	This patent	*tuf* (ef-1)
452	*Sporobolomyces salmonicolor*	This patent	*tuf* (ef-1)
453	*Sporothrix schenckii*	This patent	*tuf* (ef-1)
454	*Stephanoascus ciferrii*	This patent	*tuf* (ef-1)
455	*Trichophyton mentagrophytes*	This patent	*tuf* (ef-1)
456	*Trichosporon cutaneum*	This patent	*tuf* (ef-1)
457	*Wangiella dermatitidis*	This patent	*tuf* (ef-1)
458	*Aspergillus fumigatus*	This patent	*atpD*
459	*Blastoschizomyces capitatus*	This patent	*atpD*
460	*Candida albicans*	This patent	*atpD*
461	*Candida dubliniensis*	This patent	*atpD*
462	*Candida famata*	This patent	*atpD*
463	*Candida glabrata*	This patent	*atpD*
464	*Candida guilliermondii*	This patent	*atpD*
465	*Candida haemulonii*	This patent	*atpD*
466	*Candida inconspicua*	This patent	*atpD*
467	*Candida kefyr*	This patent	*atpD*
468	*Candida krusei*	This patent	*atpD*
469	*Candida lambica*	This patent	*atpD*
470	*Candida lusitaniae*	This patent	*atpD*
471	*Candida norvegensis*	This patent	*atpD*
472	*Candida parapsilosis*	This patent	*atpD*
473	*Candida rugosa*	This patent	*atpD*
474	*Candida sphaerica*	This patent	*atpD*
475	*Candida tropicalis*	This patent	*atpD*
476	*Candida utilis*	This patent	*atpD*
477	*Candida viswanathii*	This patent	*atpD*
478	*Candida zeylanoides*	This patent	*atpD*
479	*Coccidioides immitis*	This patent	*atpD*
480	*Cryptococcus albidus*	This patent	*atpD*
481	*Fusarium oxysporum*	This patent	*atpD*
482	*Geotrichum* spp.	This patent	*atpD*
483	*Histoplasma capsulatum*	This patent	*atpD*
484	*Malassezia furfur*	This patent	*atpD*
485	*Malassezia pachydermatis*	This patent	*atpD*
486	*Metschnikowia pulcherrima*	This patent	*atpD*
487	*Penicillium marneffei*	This patent	*atpD*
488	*Pichia anomala*	This patent	*atpD*
489	*Pichia anomala*	This pat nt	*atpD*

Table 7. Origin f th s quences in the sequence listing. (c ntinued)

SEQ ID NO.	Bacterial,fungal or parasitical species	Source	Comments*
490	*Rhodotorula minuta*	This patent	*atpD*
491	*Rhodotorula mucilaginosa*	This patent	*atpD*
492	*Sporobolomyces salmonicolor*	This patent	*atpD*
493	*Sporothrix schenckii*	This patent	*atpD*
494	*Stephanoascus ciferrii*	This patent	*atpD*
495	*Trichophyton mentagrophytes*	This patent	*atpD*
496	*Wangiella dermatitidis*	This patent	*atpD*
497	*Yarrowia lipolytica*	This patent	*atpD*
498	*Aspergillus fumigatus*	This patent	*tuf* (M)
499	*Blastoschizomyces capitatus*	This patent	*tuf* (M)
500	*Candida rugosa*	This patent	*tuf* (M)
501	*Coccidioides immitis*	This patent	*tuf* (M)
502	*Fusarium oxysporum*	This patent	*tuf* (M)
503	*Histoplasma capsulatum*	This patent	*tuf* (M)
504	*Paracoccidioides brasiliensis*	This patent	*tuf* (M)
505	*Penicillium marneffei*	This patent	*tuf* (M)
506	*Pichia anomala*	This patent	*tuf* (M)
507	*Trichophyton mentagrophytes*	This patent	*tuf* (M)
508	*Yarrowia lipolytica*	This patent	*tuf* (M)
509	*Babesia bigemina*	This patent	*tuf* (ef-1)
510	*Babesia bovis*	This patent	*tuf* (ef-1)
511	*Crithidia fasciculata*	This patent	*tuf* (ef-1)
512	*Entamoeba histolytica*	This patent	*tuf* (ef-1)
513	*Giardia lamblia*	This patent	*tuf* (ef-1)
514	*Leishmania tropica*	This patent	*tuf* (ef-1)
515	*Leishmania aethiopica*	This patent	*tuf* (ef-1)
516	*Leishmania tropica*	This patent	*tuf* (ef-1)
517	*Leishmania donovani* subsp. *donovani*	This patent	*tuf* (ef-1)
518	*Leishmania donovani* subsp. *infantum*	This patent	*tuf* (ef-1)
519	*Leishmania enriettii*	This patent	*tuf* (ef-1)
520	*Leishmania gerbilli*	This patent	*tuf* (ef-1)
521	*Leishmania hertigi* subsp. *hertigi*	This patent	*tuf* (ef-1)
522	*Leishmania major*	This patent	*tuf* (ef-1)
523	*Leishmania amazonensis*	This patent	*tuf* (ef-1)
524	*Leishmania mexicana*	This patent	*tuf* (ef-1)
525	*Leishmania tarentolae*	This patent	*tuf* (ef-1)
526	*Leishmania tropica*	This patent	*tuf* (ef-1)
527	*Neospora caninum*	This patent	*tuf* (ef-1)
528	*Trichomonas vaginalis*	This patent	*tuf* (ef-1)
529	*Trypanosoma brucei* subsp. *brucei*	This patent	*tuf* (ef-1)
530	*Crithidia fasciculata*	This patent	*atpD*
531	*Leishmania tropica*	This patent	*atpD*
532	*Leishmania aethiopica*	This patent	*atpD*
533	*Leishmania donovani* subsp. *donovani*	This patent	*atpD*
534	*Leishmania donovani* subsp. *infantum*	This patent	*atpD*
535	*Leishmania gerbilli*	This patent	*atpD*
536	*Leishmania hertigi* subsp. *hertigi*	This patent	*atpD*
537	*Leishmania major*	This patent	*atpD*
538	*Leishmania amazonensis*	This patent	*atpD*
607	*Enterococcus faecalis*	WO 98/20157	*tuf*
608	*Enterococcus faecium*	WO 98/20157	*tuf*
609	*Enterococcus gallinarum*	WO 98/20157	*tuf*
610	*Haemophilus influenzae*	Database	*tuf*
611	*Staphylococcus epidermidis*	WO 98/20157	*tuf*
612	*Salmonella choleraesuis* subsp. *choleraesuis* serotype *paratyphi* A	This patent	*tuf*
613	*Serratia ficaria*	This patent	*tuf*
614	*Enterococcus malodoratus*	This patent	*tuf* (C)
615	*Enterococcus durans*	This patent	*tuf* (C)
616	*Enterococcus pseudoavium*	This patent	*tuf* (C)
617	*Enterococcus dispar*	This patent	*tuf* (C)
618	*Enterococcus avium*	This patent	*tuf* (C)

Table 7. Origin of the sequences in the sequence listing. (continued)

SEQ ID NO.	Bacterial,fungal or parasitical species	Source	Comments*
619	*Saccharomyces cerevisiae*	Database	*tuf* (M)
621	*Enterococcus faecium*	This patent	*tuf* (C)
622	*Saccharomyces cerevisiae*	This patent	*tuf* (ef-1)
623	*Cryptococcus neoformans*	This patent	*tuf* (ef-1)
624	*Candida albicans*	This patent	*tuf* (ef-1)
662	*Corynebacterium diphtheriae*	WO 98/20157	*tuf*
663	*Candida catenulata*	This patent	*atpD*
665	*Saccharomyces cerevisiae*	Database	*tuf* (ef-1)
666	*Saccharomyces cerevisiae*	Database	*atpD*
667	*Trypanosoma cruzi*	This patent	*atpD*
668	*Corynebacterium glutamicum*	Database	*tuf*
669	*Escherichia coli*	Database	*atpD*
670	*Helicobacter pylori*	Database	*atpD*
671	*Clostridium acetobutylicum*	Database	*atpD*
672	*Cytophaga lytica*	Database	*atpD*
673	*Ehrlichia risticii*	This patent	*atpD*
674	*Vibrio cholerae*	This patent	*atpD*
675	*Vibrio cholerae*	This patent	*tuf*
676	*Leishmania enriettii*	This patent	*atpD*
677	*Babesia microtti*	This patent	*tuf* (ef-1)
678	*Cryptococcus neoformans*	This patent	*atpD*
679	*Cryptococcus neoformans*	This patent	*atpD*
680	*Cunninghamella bertholletiae*	This patent	*atpD*
684	*Candida tropicalis*	Database	*atpD* (V)
685	*Enterococcus hirae*	Database	*atpD* (V)
686	*Chlamydia pneumoniae*	Database	*atpD* (V)
687	*Halobacterium salinarum*	Database	*atpD* (V)
688	Human	Database	*atpD* (V)
689	*Plasmodium falciparum*	Database	*atpD* (V)
690	*Saccharomyces cerevisiae*	Database	*atpD* (V)
691	*Schizosaccharomyces pombe*	Database	*atpD* (V)
692	*Trypanosoma congolense*	Database	*atpD* (V)
693	*Thermus thermophilus*	Database	*atpD* (V)
698	*Escherichia coli*	Database	*tuf*
709	*Borrelia burgdorferi*	genome project	*atpD* (V)
710	*Treponema pallidum*	genome project	*atpD* (V)
711	*Chlamydia trachomatis*	genome project	*atpD* (V)
712	*Enterococcus faecalis*	genome project	*atpD* (V)
713	*Methanosarcina barkeri*	Database	*atpD* (V)
714	*Methanosarcina jannaschii*	Database	*atpD* (V)
715	*Porphyromonas gingivalis*	genome project	*atpD* (V)
716	*Streptococcus pneumoniae*	genome project	*atpD* (V)
717	*Burkholderia mallei*	This patent	*tuf*
718	*Burkholderia pseudomallei*	This patent	*tuf*
719	*Clostridium beijerincki*	This patent	*tuf*
720	*Clostridium innocuum*	This patent	*tuf*
721	*Clostridium novyi*	This patent	*tuf*
722	*Clostridium septicum*	This patent	*tuf*
723	*Clostridium tertium*	This patent	*tuf*
724	*Clostridium tetani*	This patent	*tuf*
725	*Enterococcus malodoratus*	This patent	*tuf*
726	*Enterococcus sulfureus*	This patent	*tuf*
727	*Lactococcus garvieae*	This patent	*tuf*
728	*Mycoplasma pirum*	This patent	*tuf*
729	*Mycoplasma salivarium*	This patent	*tuf*
730	*Neisseria polysaccharea*	This patent	*tuf*
731	*Salmonella choleraesuis* subsp. *choleraesuis* serotype *enteritidis*	This patent	*tuf*
732	*Salmonella choleraesuis* subsp. *choleraesuis* serotype *gallinarum*	This patent	*tuf*
733	*Salmonella choleraesuis* subsp. *choleraesuis* serotype *paratyphi B*	This patent	*tuf*
734	*Salmonella choleraesuis* subsp. *choleraesuis* serotype *virchow*	This patent	*tuf*
735	*Serratia grimesii*	This patent	*tuf*
736	*Clostridium difficile*	This patent	*tuf*
737	*Burkholderia pseudomallei*	This patent	*atpD*

Table 7. Origin f the sequenc s in th sequence listing. (continued)

SEQ ID NO.	Bacterial,fungal or parasitical species	Source	Comments*
738	*Clostridium bifermentans*	This pat nt	*atpD*
739	*Clostridium beijerinckii*	This patent	*atpD*
740	*Clostridium difficile*	This patent	*atpD*
741	*Clostridium ramosum*	This patent	*atpD*
742	*Clostridium septicum*	This patent	*atpD*
743	*Clostridium tertium*	This patent	*atpD*
744	*Comamonas acidovorans*	This patent	*atpD*
745	*Klebsiella pneumoniae* subsp. *rhinoscleromatis*	This patent	*atpD*
746	*Neisseria canis*	This patent	*atpD*
747	*Neisseria cinerea*	This patent	*atpD*
748	*Neisseria cuniculi*	This patent	*atpD*
749	*Neisseria elongata* subsp. *elongata*	This patent	*atpD*
750	*Neisseria flavescens*	This patent	*atpD*
751	*Neisseria gonorrhoeae*	This patent	*atpD*
752	*Neisseria gonorrhoeae*	This patent	*atpD*
753	*Neisseria lactamica*	This patent	*atpD*
754	*Neisseria meningitidis*	This patent	*atpD*
755	*Neisseria mucosa*	This patent	*atpD*
756	*Neisseria subflava*	This patent	*atpD*
757	*Neisseria weaveri*	This patent	*atpD*
758	*Neisseria animalis*	This patent	*atpD*
759	*Proteus penneri*	This patent	*atpD*
760	*Salmonella choleraesuis* subsp. *choleraesuis* serotype *enteritidis*	This patent	*atpD*
761	*Yersinia pestis*	This patent	*atpD*
762	*Burkholderia mallei*	This patent	*atpD*
763	*Clostridium sordellii*	This patent	*atpD*
764	*Clostridium novyi*	This patent	*atpD*
765	*Clostridium botulinum*	This patent	*atpD*
766	*Clostridium histolyticum*	This patent	*atpD*
767	*Peptostreptococcus prevotii*	This patent	*atpD*
768	*Absidia corymbifera*	This patent	*atpD*
769	*Alternaria alternata*	This patent	*atpD*
770	*Aspergillus flavus*	This patent	*atpD*
771	*Mucor circinelloides*	This patent	*atpD*
772	*Piedraia hortai*	This patent	*atpD*
773	*Pseudallescheria boydii*	This patent	*atpD*
774	*Rhizopus oryzae*	This patent	*atpD*
775	*Scopulariopsis koningii*	This patent	*atpD*
776	*Trichophyton mentagrophytes*	This patent	*atpD*
777	*Trichophyton tonsurans*	This patent	*atpD*
778	*Trichosporon cutaneum*	This patent	*atpD*
779	*Cladophialophora carrionii*	This patent	*tuf* (ef-1)
780	*Cunninghamella bertholletiae*	This patent	*tuf* (ef-1)
781	*Curvularia lunata*	This patent	*tuf* (ef-1)
782	*Fonsecaea pedrosoi*	This patent	*tuf* (ef-1)
783	*Microsporum audouinii*	This patent	*tuf* (ef-1)
784	*Mucor circinelloides*	This patent	*tuf* (ef-1)
785	*Phialaphora verrucosa*	This patent	*tuf* (ef-1)
786	*Saksenaea vasiformis*	This patent	*tuf* (ef-1)
787	*Syncephalastrum racemosum*	This patent	*tuf* (ef-1)
788	*Trichophyton tonsurans*	This patent	*tuf* (ef-1)
789	*Trichophyton mentagrophytes*	This patent	*tuf* (ef-1)
790	*Bipolaris hawaiiensis*	This patent	*tuf* (ef-1)
791	*Aspergillus fumigatus*	This patent	*tuf* (M)
792	*Trichophyton mentagrophytes*	This patent	*tuf* (M)
827	*Clostridium novyi*	This patent	*atpD* (V)
828	*Clostridium difficile*	This patent	*atpD* (V)
829	*Clostridium septicum*	This patent	*atpD* (V)
830	*Clostridium botulinum*	This patent	*atpD* (V)
831	*Clostridium perfringens*	This patent	*atpD* (V)
832	*Clostridium tetani*	This patent	*atpD* (V)

Tabl 7. Origin of the sequenc s in the sequence listing. (continued)

SEQ ID NO.	Bacterial,fungal or parasitical species	Source	Comments*
833	*Streptococcus pyogenes*	Database	*atpD* (V)
834	*Babesia bovis*	This patent	*atpD* (V)
835	*Cryptosporidium parvum*	This patent	*atpD* (V)
836	*Leishmania donovani* subsp. *infantum*	This patent	*atpD* (V)
837	*Leishmania major*	This patent	*atpD* (V)
838	*Leishmania tarentolae*	This patent	*atpD* (V)
839	*Trypanosoma brucei*	This patent	*atpD* (V)
840	*Trypanosoma cruzi*	This patent	*tuf* (ef-1)
841	*Trypanosoma cruzi*	This patent	*tuf* (ef-1)
842	*Trypanosoma cruzi*	This patent	*tuf* (ef-1)
843	*Babesia bovis*	This patent	*tuf* (M)
844	*Leishmania aethiopica*	This patent	*tuf* (M)
845	*Leishmania amazonensis*	This patent	*tuf* (M)
846	*Leishmania donovani* subsp. *donovani*	This patent	*tuf* (M)
847	*Leishmania donovani* subsp. *infantum*	This patent	*tuf* (M)
848	*Leishmania enriettii*	This patent	*tuf* (M)
849	*Leishmania gerbilli*	This patent	*tuf* (M)
850	*Leishmania major*	This patent	*tuf* (M)
851	*Leishmania mexicana*	This patent	*tuf* (M)
852	*Leishmania tarentolae*	This patent	*tuf* (M)
853	*Trypanosoma cruzi*	This patent	*tuf* (M)
854	*Trypanosoma cruzi*	This patent	*tuf* (M)
855	*Trypanosoma cruzi*	This patent	*tuf* (M)
856	*Babesia bigemina*	This patent	*atpD*
857	*Babesia bovis*	This patent	*atpD*
858	*Babesia microtti*	This patent	*atpD*
859	*Leishmania guyanensis*	This patent	*atpD*
860	*Leishmania mexicana*	This patent	*atpD*
861	*Leishmania tropica*	This patent	*atpD*
862	*Leishmania tropica*	This patent	*atpD*
863	*Bordetella pertussis*	Database	*tuf*
864	*Trypanosoma brucei*	Database	*tuf* (ef-1)
865	*Cryptosporidium parvum*	This patent	*tuf* (ef-1)
866	*Staphylococcus saprophyticus*	This patent	*atpD*
867	*Zoogloea ramigera*	This patent	*atpD*
868	*Staphylococcus saprophyticus*	This patent	*tuf*
869	*Enterococcus casseliflavus*	This patent	*tuf*
870	*Enterococcus casseliflavus*	This patent	*tuf*
871	*Enterococcus flavescens*	This patent	*tuf*
872	*Enterococcus gallinarum*	This patent	*tuf*
873	*Enterococcus gallinarum*	This patent	*tuf*
874	*Staphylococcus haemolyticus*	This patent	*tuf*
875	*Staphylococcus epidermidis*	This patent	*tuf*
876	*Staphylococcus epidermidis*	This patent	*tuf*
877	*Staphylococcus epidermidis*	This patent	*tuf*
878	*Staphylococcus epidermidis*	This patent	*tuf*
879	*Enterococcus gallinarum*	This patent	*tuf*
880	*Pseudomonas aeruginosa*	This patent	*tuf*
881	*Enterococcus casseliflavus*	This patent	*tuf*
882	*Enterococcus casseliflavus*	This patent	*tuf*
883	*Enterococcus faecalis*	This patent	*tuf*
884	*Enterococcus faecalis*	This patent	*tuf*
885	*Enterococcus faecium*	This patent	*tuf*
886	*Enterococcus faecium*	This patent	*tuf*
887	*Zoogloea ramigera*	This patent	*tuf*
888	*Enterococcus faecalis*	This patent	*tuf*
889	*Aspergillus fumigatus*	This patent	*atpD*
890	*Penicillium marneffei*	This patent	*atpD*
891	*Paecilomyces lilacinus*	This patent	*atpD*
892	*Penicillium marneffei*	This patent	*atpD*
893	*Sporothrix sch nckii*	This patent	*atpD*

Table 7. Origin of the sequences in the sequence listing. (continued)

SEQ ID NO.	Bacterial,fungal or parasitical species	Source	Comments*
894	*Malbranchea filamentosa*	This patent	*atpD*
895	*Paecilomyces lilacinus*	This patent	*atpD*
896	*Aspergillus niger*	This patent	*atpD*
897	*Aspergillus fumigatus*	This patent	*tuf* (ef-1)
898	*Penicillium marneffei*	This patent	*tuf* (ef-1)
899	*Piedraia hortai*	This patent	*tuf* (ef-1)
900	*Paecilomyces lilacinus*	This patent	*tuf* (ef-1)
901	*Paracoccidioides brasiliensis*	This patent	*tuf* (ef-1)
902	*Sporothrix schenckii*	This patent	*tuf* (ef-1)
903	*Penicillium marneffei*	This patent	*tuf* (ef-1)
904	*Curvularia lunata*	This patent	*tuf* (M)
905	*Aspergillus niger*	This patent	*tuf* (M)
906	*Bipolaris hawaiiensis*	This patent	*tuf* (M)
907	*Aspergillus flavus*	This patent	*tuf* (M)
908	*Alternaria alternata*	This patent	*tuf* (M)
909	*Penicillium marneffei*	This patent	*tuf* (M)
910	*Penicillium marneffei*	This patent	*tuf* (M)
918	*Escherichia coli*	Database	*recA*
929	*Bacteroides fragilis*	This patent	*atpD* (V)
930	*Bacteroides distasonis*	This patent	*atpD* (V)
931	*Porphyromonas asaccharolytica*	This patent	*atpD* (V)
932	*Listeria monocytogenes*	This patent	*tuf*
939	*Saccharomyces cerevisiae*	Database	*rad51*
940	*Saccharomyces cerevisiae*	Database	*dmc1*

* *atpD* indicates *atpD* sequences ot the F-type

atpD (V) indicates *atpD* sequences of the V-Type

tuf indicates *tuf* sequences

tuf (C) indicates *tuf* sequences divergent from main (usually A and B) copies of the elongation factor-Tu

tuf (ef-1) indicates *tuf* sequences of the eukaryotic type (elongation factor 1α)

tuf (M) indicates *tuf* sequences from organellar (mostly mitochondrial) origin

recA indicates *recA* sequences, *Rad51* indicates *Rad51* sequences or *rad51* homologs and *dmc1* indicates *dmc1* sequences or *dmc1* homologs

Table 8. Bacterial species used to test the specificity of the *Streptococcus agalactiae*-specific amplification primers from *tuf* sequence.

Strain	Reference number	Strain	Reference number
Streptococcus acidominimus	ATCC 51726	*Bacteroides caccae*	ATCC 43185
Streptococcus agalactiae	ATCC 12403	*Bacteroides vulgatus*	ATCC 8482
Streptococcus agalactiae	ATCC 12973	*Bacteroides fragilis*	ATCC 25285
Streptococcus agalactiae	ATCC 13813	*Candida albicans*	ATCC 11006
Streptococcus agalactiae	ATCC 27591	*Clostridium innoculum*	ATCC 14501
Streptococcus agalactiae	CDCs 1073	*Clostridium ramosum*	ATCC 25582
Streptococcus anginosus	ATCC 27335	*Lactobacillus casei* subsp. *casei*	ATCC 393
Streptococcus anginosus	ATCC 33397	*Clostridium septicum*	ATCC 12464
Streptococcus bovis	ATCC 33317	*Corynebacterium cervicis*	NCTC 10604
Streptococcus anginosus	ATCC 27823	*Corynebacterium genitalium*	ATCC 33031
Streptococcus cricetus	ATCC 19642	*Corynebacterium urealyticum*	ATCC 43042
Streptococcus cristatus	ATCC 51100	*Enterococcus faecalis*	ATCC 29212
Streptococcus downei	ATCC 33748	*Enterococcus faecium*	ATCC 19434
Streptococcus dysgalactiae	ATCC 43078	*Eubacterium lentum*	ATCC 43055
Streptococcus equi subsp *equi*	ATCC 9528	*Eubacterium nodutum*	ATCC 33099
Streptococcus ferus	ATCC 33477	*Gardnerella vaginalis*	ATCC 14018
Streptococcus gordonii	ATCC 10558	*Lactobacillus acidophilus*	ATCC 4356
Streptococcus macacae	ATCC 35911	*Lactobacillus crispatus*	ATCC 33820
Streptococcus mitis	ATCC 49456	*Lactobacillus gasseri*	ATCC 33323
Streptococcus mutans	ATCC 25175	*Lactobacillus johnsonii*	ATCC 33200
Streptococcus oralis	ATCC 35037	*Lactococcus lactis* subsp. *lactis*	ATCC 19435
Streptococcus parasanguinis	ATCC 15912	*Lactococcus lactis* subsp. *lactis*	ATCC 11454
Streptococcus parauberis	DSM 6631	*Listeria innocua*	ATCC 33090
Streptococcus pneumoniae	ATCC 27336	*Micrococcus luteus*	ATCC 9341
Streptococcus pyogenes	ATCC 19615	*Escherichia coli*	ATCC 25922
Streptococcus ratti	ATCC 19645	*Micrococcus lylae*	ATCC 27566
Streptococcus salivarius	ATCC 7073	*Porphyromonas asaccharolytica*	ATCC 25260
Streptococcus sanguinis	ATCC 10556	*Prevotella corporis*	ATCC 33547
Streptococcus sobrinus	ATCC 27352	*Prevotella melanogenica*	ATCC 25845
Streptococcus suis	ATCC 43765	*Staphylococcus aureus* subsp. *aureus*	ATCC 13301
Streptococcus uberis	ATCC 19436	*Staphylococcus epidermidis*	ATCC 14990
Streptococcus vestubularis	ATCC 49124	*Staphylococcus saprophyticus*	ATCC 15305

Table 9. Bacterial species used to test the specificity of the *Streptococcus agalactiae*-specific amplification primers from *atpD* sequence.

Strain	Reference number	Strain	Reference number
Streptococcus acidominimus	ATCC 51726	*Streptococcus gordonii*	ATCC 10558
Streptococcus agalactiae	ATCC 12400	*Streptococcus macacae*	ATCC 35911
Streptococcus agalactiae	ATCC 12403	*Streptococcus mitis*	ATCC 49456
Streptococcus agalactiae	ATCC 12973	*Streptococcus mutans*	ATCC 25175
Streptococcus agalactiae	ATCC 13813	*Streptococcus oralis*	ATCC 35037
Streptococcus agalactiae	ATCC 27591	*Streptococcus parasanguinis*	ATCC 15912
Streptococcus agalactiae	CDCs-1073	*Streptococcus parauberis*	DSM 6631
Streptococcus anginosus	ATCC 27335	*Streptococcus pneumoniae*	ATCC 27336
Streptococcus anginosus	ATCC 27823	*Streptococcus pyogenes*	ATCC 19615
Streptococcus bovis	ATCC 33317	*Streptococcus ratti*	ATCC 19645
Streptococcus cricetus	ATCC 19642	*Streptococcus salivarius*	ATCC 7073
Streptococcus cristatus	ATCC 51100	*Streptococcus sanguinis*	ATCC 10556
Streptococcus downei	ATCC 33748	*Streptococcus sobrinus*	ATCC 27352
Streptococcus dysgalactiae	ATCC 43078	*Streptococcus suis*	ATCC 43765
Streptococcus equi subsp. *equi*	ATCC 9528	*Streptococcus uberis*	ATCC 19436
Streptococcus ferus	ATCC 33477	*Streptococcus vestibularis*	ATCC 49124

Table 10. Bacterial species used to test the specificity f the *Enterococcus* genus-specific amplificati n primers from *tuf* sequenc .

Strain	Refer nce number	PCR result
Abiotrophia adjacens	ATCC 49175	positive
Abiotrophia defectiva	ATCC 49176	negative
Acinetobacter baumannii	ATCC 19606	negative
Bordetella pertussis	ATCC 9797	negative
Branhamella catarrhalis	ATCC 43628	negative
Bulkholderia cepacia	LSPQ 2217	negative
Citrobacter freundii	ATCC 8090	negative
Corynebacterium aquaticus	ATCC 14665	negative
Enterobacter cloacae	ATCC 13047	negative
Enterococcus avium	ATCC 14025	positive
Enterococcus casseliflavus	ATCC 25788	positive
Enterococcus casseliflavus	R689	positive
Enterococcus casseliflavus	R754	positive
Enterococcus casseliflavus	R763	positive
Enterococcus cecorum	ATCC 43198	positive
Enterococcus columbae	ATCC 51263	positive
Enterococcus dispar	ATCC 51266	positive
Enterococcus durans	ATCC 19432	positive
Enterococcus faecalis	ATCC 51299	positive
Enterococcus faecalis	R422	positive
Enterococcus faecalis	R485	positive
Enterococcus faecalis	R498	positive
Enterococcus faecalis	R503	positive
Enterococcus faecalis	R575	positive
Enterococcus faecalis	R577	positive
Enterococcus faecalis	R610	positive
Enterococcus faecalis	R617	positive
Enterococcus faecalis	ATCC 29212	positive
Enterococcus faecium	ATCC 19434	positive
Enterococcus faecium	ATCC 19434	positive
Enterococcus faecium	ATCC 700221	positive
Enterococcus faecium	R421	positive
Enterococcus faecium	R446	positive
Enterococcus faecium	R448	positive
Enterococcus faecium	R449	positive
Enterococcus faecium	R450	positive
Enterococcus faecium	R481	positive
Enterococcus faecium	R482	positive
Enterococcus faecium	R494	positive
Enterococcus faecium	R648	positive
Enterococcus flavescens	ATCC 49996	positive
Enterococcus flavescens	R758	positive
Enterococcus flavescens	R760	positive
Enterococcus gallinarum	ATCC 49573	positive
Enterococcus gallinarum	LSPQ 3364	positive
Enterococcus gallinarum	R420	positive
Enterococcus gallinarum	R431	positive
Enterococcus gallinarum	R432	positive
Enterococcus gallinarum	R631	positive
Enterococcus gallinarum	R684	positive
Enterococcus gallinarum	R691	positive
Enterococcus gallinarum	R757	positive
Enterococcus gallinarum	R764	positive

Table 10. Bacterial species used to test the specificity of the *Enterococcus* g nus-specific amplification primers from *tuf* sequence (c ntinued).

Strain	Reference number	PCR result
Enterococcus hirae	ATCC 8043	positive
Enterococcus malodoratus	ATCC 43197	positive
Enterococcus mundtii	ATCC 43186	positive
Enterococcus pseudoavium	ATCC 49372	positive
Enterococcus raffinosus	ATCC 49427	positive
Enterococcus saccharolyticus	ATCC 43076	positive
Enterococcus solitarius	ATCC 49428	positive
Escherichia coli	ATCC 25922	negative
Gemella haemolysans	ATCC 10379	positive
Haemophilus influenzae	ATCC 9007	negative
Hafnia alvei	ATCC 13337	negative
Kingella kingae	ATCC 23330	negative
Klebsiella oxytoca	ATCC 13182	negative
Lactobacillus acidophilus	ATCC 4356	negative
Listeria grayi	ATCC 19120	negative
Listeria innocua	ATCC 33090	negative
Listeria ivanovii	ATCC 19119	negative
Listeria monocytogenes	ATCC 15313	negative
Listeria monocytogenes	ATCC 35152	negative
Listeria monocytogenes	BD1427	negative
Listeria monocytogenes	L 279	negative
Listeria monocytogenes	L 374	negative
Listeria monocytogenes	L9	negative
Listeria monocytogenes	LSPQ 5093202	negative
Listeria monocytogenes	SS2	negative
Listeria seeligeri	ATCC 35967	negative
Micrococcus luteus	ATCC 9341	negative
Morganella morganii subsp. *morganii*	ATCC 25830	negative
Neisseria meningitidis	ATCC 13077	negative
Pasteurella aerogenes	ATCC 27883	negative
Proteus vulgaris	ATCC 13315	negative
Providencia alcalifaciens	ATCC 9886	negative
Providencia rettgeri	ATCC 9250	negative
Pseudomonas aeruginosa	ATCC 27853	negative
Salmonella typhimurium	ATCC 14028	negative
Serratia marcescens	ATCC 13880	negative
Shigella flexneri	ATCC 12022	negative
Shigella sonnei	ATCC 29930	negative
Staphylococcus aureus subsp. *aureus*	ATCC 43300	negative
Staphylococcus capitis subsp.*capitis*	ATCC 27840	negative
Staphylococcus epidermidis	ATCC 14990	negative
Staphylococcus haemolyticus	ATCC 29970	negative
Staphylococcus hominis subsp. *hominis*	ATCC 27844	negative
Staphylococcus lugdunensis	ATCC 43809	negative
Staphylococcus saprophyticus	ATCC 15305	negative
Staphylococcus simulans	ATCC 27848	negative
Staphylococcus warneri	ATCC 27836	negative
Streptococcus anginosus	ATCC 27335	negative
Streptococcus anginosus	ATCC 27823	negative
Streptococcus anginosus	ATCC 33397	negative
Streptococcus bovis	ATCC 33317	negative
Streptococcus cristatus	ATCC 51100	negative
Streptococcus mitis	ATCC49456	negative
Streptococcus mutans	ATCC 25175	negative

Table 10. Bacterial species used to test the specificity f the *Ent rococcus* genus-specific amplification primers fr m *tuf* sequence (continued).

Strain	Refer nc number	PCR result
Streptococcus parasanguinis	ATCC 15912	negative
Streptococcus pneumoniae	ATCC 27736	negative
Streptococcus pneumoniae	ATCC 6303	negative
Streptococcus pyogenes	ATCC 19615	negative
Streptococcus salivarius	ATCC 7073	negative
Streptococcus sanguinis	ATCC 10556	negative
Streptococcus suis	ATCC 43765	negative
Yersinia enterocolitica	ATCC 9610	negative

Table 11. Microbial species for which *atpD* and/or *tuf* and/or *recA* and/or *Rad51* and/or *dmc1* sequences are available in public databases

Species	Strain	Accession number	Coding gene*
Bacteria			
Agrobacterium tumefaciens		X99673	*tuf*
Agrobacterium tumefaciens		X99674	*tuf*
Anacystis nidulans	PCC 6301	X17442	*tuf*
Aquifex aeolicus	VF5	AE000669	*tuf*
Aquifex pyrophilus		Y15787	*tuf*
Bacillus halodurans	C-125	AB017508	*tuf*
Bacillus stearothermophilus	CCM 2184	AJ000260	*tuf*
Bacillus subtilis	DSM 10	Z99104	*tuf*
Bacillus subtilis	168	D64127	*tuf*
Bacteroides fragilis	DSM 1151	-[1]	*tuf*
Bordetella bronchiseptica	RB50	Genome project[2]	*tuf*
Bordetella pertussis	Tohama 1	Genome project[2]	*tuf*
Borrelia burdorgferi	B31	U78193	*tuf*
Brevibacterium linens	DSM 20425	X76863	*tuf*
Buchnera aphidicola	Ap	Y12307	*tuf*
Campylobacter jejuni	NCTC 11168	Y17167	*tuf*
Chlamydia pneumoniae	CWL029	AE001592	*tuf*
Chlamydia trachomatis	F/IC-Cal-13	L22216	*tuf*
Chlamydia trachomatis	D/WW-3/CX	AE001305	*tuf*
Chlamydia trachomatis		M74221	*tuf*
Chlorobium vibrioforme	DSM 263	X77033	*tuf*
Chloroflexus aurantiacus	DSM 636	X76865	*tuf*
Clostridium acetobutylicum	ATCC 824	Genome project[2]	*tuf*
Clostridium difficile	630	Genome project[2]	*tuf*
Corynebacterium glutamicum	ASO 19	X77034	*tuf*
Cytophaga lytica	DSM 2039	X77035	*tuf*
Deinonema spp.		-[1]	*tuf*
Eikenella corrodens	ATCC 23834	Z12610	*tuf*
Escherichia coli		J01690	*tuf*
Escherichia coli	K-12 MG1655	U00096	*tuf*
Escherichia coli		J01717	*tuf*
Escherichia coli	K-12 MG1655	U00006	*tuf*
Escherichia coli		X57091	*tuf*
Fervidobacterium islandicum	DSM 5733	Y15788	*tuf*
Fibrobacter succinogenes	S85	X76866	*tuf*
Flavobacterium ferrigeneum	DSM 13524	X76867	*tuf*
Flexistipes sinusarabici		X59461	*tuf*
Gloeobacter violaceus	PCC 7421	U09433	*tuf*
Gloeothece spp.	PCC 6501	U09434	*tuf*
Haemophilus actinomycetemcomitans	HK1651	Genome project[2]	*tuf*
Haemophilus influenzae	RD	L42023	*tuf*
Haloarcula marismortui		X16677	*tuf*
Helicobacter pylori	26695	AE000511	*tuf*
Helicobacter pylori	J99	AE001541	*tuf*
Herpetosiphon aurantiacus	Hpga1	X76868	*tuf*
Lactobacillus paracasei		E13922	*tuf*
Methanobacterium thermoautrophicum	delta H	AE000877	*tuf*
Methanococcus jannaschii	ATCC 43067	U67486	*tuf*
Methanococcus vannielii		X05698	*tuf*

Table 11. Microbial species for which *atpD* and/or *tuf* and/or *recA* and/or *Rad51* and/or *dmc1* s quences ar available in public databases (continued)

Species	Strain	Accession number	Coding gene*
Micrococcus luteus	IFO 3333	M17788	*tuf*
Mycobacterium avium	104	Genome project[2]	*tuf*
Mycobacterium bovis	AF2122/97	Genome project[2]	*tuf*
Mycobacterium leprae	Thai 53	D13869	*tuf*
Mycobacterium leprae		Z14314	*tuf*
Mycobacterium leprae		L13276	*tuf*
Mycobacterium tuberculosis	H37Rv	Z84395	*tuf*
Mycobacterium tuberculosis	Erdmann	S40925	*tuf*
Mycobacterium tuberculosis		AD000005	*tuf*
Mycoplasma capricolum	PG-31	X16462	*tuf*
Mycoplasma genitalium	G37	U39732	*tuf*
Mycoplasma hominis		X57136	*tuf*
Mycoplasma hominis	PG21	M57675	*tuf*
Mycoplasma pneumoniae	M129	AE000019	*tuf*
Neisseria gonorrhoeae	MS11	L36380	*tuf*
Neisseria meningitidis	Z2491	Genome project[2]	*tuf*
Peptococcus niger	DSM 20745	X76869	*tuf*
Phophyromonas gingivalis	W83	Genome project[2]	*tuf*
Phormidium ectocarpi	PCC 7375	U09443	*tuf*
Planobispora rosea	ATCC 53773	U67308	*tuf*
Plectonema boryanum	PCC 73110	U09444	*tuf*
Prochlorothrix hollandica		U09445	*tuf*
Pseudomonas aeruginosa	PAO-1	Genome project[2]	*tuf*
Rickettsia prowazekii	Madrid E	AJ235272	*tuf*
Salmonella typhimurium	LT2 trpE91	X55116	*tuf*
Salmonella typhimurium	LT2 trpE91	X55117	*tuf*
Serratia marcescens		AF058451	*tuf*
Serpulina hyodysenteriae	B204	U51635	*tuf*
Shewanella putida		Genome project[2]	*tuf*
Shewanella putrefaciens	DSM 50426	-[1]	*tuf*
Shewanella putrefaciens	MR-1	Genome project[2]	*tuf*
Spirochaeta aurantia	DSM 1902	X76874	*tuf*
Stigmatella aurantiaca	DW4	X82820	*tuf*
Stigmatella aurantiaca	Sg a1	X76870	*tuf*
Streptococcus mutans	*UAB159*	*Genome project2*	*tuf*
Streptococcus mutans	GS-5 Kuramitsu	U75481	*tuf*
Streptococcus oralis	NTCC 11427	P331701	*tuf*
Streptococcus pyogenes	M1-GAS	Genome project[2]	*tuf*
Streptomyces aureofaciens	ATCC 10762	AF007125	*tuf*
Streptomyces cinnamoneus	Tue89	X98831	*tuf*
Streptomyces coelicolor	M145	X77039	*tuf*
Streptomyces collinus	BSM 40733	S79408	*tuf*
Streptomyces ramocissimus		X67057	*tuf*
Streptomyces ramocissimus		X67058	*tuf*
Synechocystis spp.	PCC 6803	D90913	*tuf*
Taxeobacter occealus	Myx 2105	X77036	*tuf*
Thermoplasma acidophilum	DSM 1728	X53866	*tuf*
Thermotoga maritima		M27479	*tuf*
Thermus aquaticus	EP 00276	X66322	*tuf*
Thermus thermophilus	HB8	X06657	*tuf*
Thermus thermophilus	HB8	X05977	*tuf*
Thiobacillus cuprinus	Hoe5	X76871	*tuf*
Thiobacillus cuprinus	DSM 5495	U78300	*tuf*

Table 11. Microbial species for which *atpD* and/or *tuf* and/or *recA* and/or *Rad51* and/or *dmc1* sequences are available in public databases (c ntinued)

Species	Strain	Accession number	Coding gene*
Treponema pallidum		AE001202	*tuf*
Ureaplasma urealyticum	ATCC 33697	Z34275	*tuf*
Vibrio cholerae	N16961	TIGR2	*tuf*
Wolinella succinogenes	DSM 1740	X76872	*tuf*
Yersinia pestis	CO-92	Genome project[2]	*tuf*
Fungi			
Saccharomyces cerevisiae		K00428	*tuf* (M)
Absidia glauca	CBS 101.48	X54730	*tuf* (ef-1)
Arxula adeninivorans	Ls3	Z47379	*tuf* (ef-1)
Aspergillus oryzae	KBN616	AB007770	*tuf* (ef-1)
Aureobasidium pullulans	R106	U19723	*tuf* (ef-1)
Candida albicans	SC5314	M29935	*tuf* (ef-1)
Candida albicans	SC5314	M29934	*tuf* (ef-1)
Cryptococcus neoformans	B3501	U81803	*tuf* (ef-1)
Cryptococcus neoformans	M1-106	U81804	*tuf* (ef-1)
Eremothecium gossypii	ATCC 10895	X73978	*tuf* (ef-1)
Fusarium oxysporum	NRRL 26037	AF008498	*tuf* (ef-1)
Histoplasma capsulatum	186AS	U14100	*tuf* (ef-1)
Podospora anserina		X74799	*tuf* (ef-1)
Puccinia graminis	race 32	X73529	*tuf* (ef-1)
Rhizomucor racemosus	ATCC 1216B	J02605	*tuf* (ef-1)
Rhizomucor racemosus	ATCC 1216B	X17476	*tuf* (ef-1)
Rhizomucor racemosus	ATCC 1216B	X17475	*tuf* (ef-1)
Rhodotorula mucilaginosa		AF016239	*tuf* (ef-1)
Saccharomyces cerevisiae		X01638	*tuf* (ef-1)
Saccharomyces cerevisiae		X00779	*tuf* (ef-1)
Schizosaccharomyces pombe		U42189	*tuf* (ef-1)
Trichoderma reesei	QM9414	Z23012	*tuf* (ef-1)
Yarrowia lipolytica		AF054510	*tuf* (ef-1)
Parasites			
Blastocystis hominis	HE87-1	D64080	*tuf* (ef-1)
Giardia lamblia		D14342	*tuf* (ef-1)
Kentrophoros spp.		AF056101	*tuf* (ef-1)
Leishmania amazonensis	IFLA/BR/67/PH8	M92653	*tuf* (ef-1)
Leishmania braziliensis		U72244	*tuf* (ef-1)
Onchocerca volvulus		M64333	*tuf* (ef-1)
Porphyra purpurea	Avonport	U08844	*tuf* (ef-1)
Plasmodium berghei	ANKA	AJ224150	*tuf* (ef-1)
Plasmodium falciparum	K1	X60488	*tuf* (ef-1)
Plasmodium knowlesi	line H	AJ224153	*tuf* (ef-1)
Toxoplasma gondii	RH	Y11431	*tuf* (ef-1)
Trypanosoma cruzi	Y	L76077	*tuf* (ef-1)
Trypanosoma brucei	LVH/75/ USAMRU-K/18	U10562	*tuf* (ef-1)

Table 11. Microbial species for which *atpD* and/or *tuf* and/or *recA* and/or *Rad51* and/or *dmc1* sequences are available in public databases (continued)

Species	Strain	Accession number	Coding gene*
Bacteria			
Acetobacterium woodi	DSM 1030	U10505	*atpD*
Bacillus caldotenax		D38058	*atpD*
Bacillus firmus	OF4	M60117	*atpD*
Bacillus megaterium	QM B1551	M20255	*atpD*
Bacillus stearothermophilus	IFO1035	D38060	*atpD*
Bacillus subtilis	168	Z28592	*atpD*
Bacteroides fragilis	DSM 2151	M22247	*atpD*
Bordetella bronchiseptica	RB50	Genome project[2]	*atpD*
Bordetella pertussis	Tohama 1	Genome project[2]	*atpD*
Borrelia burgdorferi		Genome project[2]	*atpD* (V)
Burkholderia cepacia	DSM50181	X76877	*atpD*
Brevibacterium flavum	MJ-233	E09634	*atpD*
Campylobacter jejuni	NCTC 11168	Genome project[2]	*atpD*
Chlamydia pneumoniae		Genome project[2]	*atpD* (V)
Chlamydia trachomatis	MoPn	Genome project[2]	*atpD* (V)
Chlorobium vibrioforme	DSM 263	X76873	*atpD*
Citrobacter freundii	JEO503	AF037156	*atpD*
Clostridium acetobutylicum	ATCC 824	Genome project[2]	*atpD*
Clostridium acetobutylicum	DSM 792	AF101055	*atpD*
Clostridium difficile	630	Genome project2	*atpD*
Corynebacterium glutamicum	ASO 19	X76875	*atpD*
Corynebacterium glutamicum	DSM 792	AF101055	*atpD*
Cytophaga lytica	DSM 2039	M22535	*atpD*
Enterobacter aerogenes	DSM 30053	-[3]	*atpD*
Enterococcus faecalis		M90060	*atpD*
Enterococcus faecalis	V583	Genome project[2]	*atpD* (V)
Enterococcus hirae	ATCC 9790	D17462	*atpD* (V)
Escherichia coli		V00267	*atpD*
Escherichia coli		J01594	*atpD*
Escherichia coli	K12 MG1655	L10328	*atpD*
Escherichia coli		V00311	*atpD*
Escherichia coli		*M25464*	*atpD*
Flavobacterium ferrugineum	*DSM 13524*	*-3*	*atpD*
Haemophilus actinomycetemcomitans		*Genome project2*	*atpD*
Haemophilus influenzae	Rd	U32730	*atpD*
Halobacterium salinarum		S56356	*atpD* (V)
Haloferax volcanii	WR 340	X79516	*atpD*
Helicobacter pylori	NCTC 11638	AF004014	*atpD*
Lactobacillus casei	DSM 20021	X64542	*atpD*
Methanococcus jannaschii	DSM 2661	U67477	*atpD* (V)
Methanosarcina barkeri	DSM 800	J04836	*atpD* (V)
Moorella thermoacetica	ATCC 39073	U64318	*atpD*
Mycobacterium avium	104	Genome project[2]	*atpD*
Mycobacterium bovis	AF2122/97	Genome project[2]	*atpD*
Mycobacterium leprae		U15186	*atpD*
Mycobacterium tuberculosis	H37Rv	Z73419	*atpD*
Mycoplasma gallisepticum		X64256	*atpD*

Table 11. Microbial species for which *atpD* and/or *tuf* and/or *recA* and/or *Rad51* and/or *dmc1* sequences are available in public databases (continued)

Species	Strain	Accession number	Coding gene*
Mycoplasma genitalium	G37	U39725	*atpD*
Mycoplasma pneumoniae	M129	U43738	*atpD*
Neisseria gonorrhoeae	FA 1090	Genome project[2]	*atpD*
Neisseria meningitidis	Z2491	Genome project[2]	*atpD*
Peptococcus niger	DSM 20475	X76878	*atpD*
Pectinatus frisingensis	DSM 20465	X64543	*atpD*
Pirellula marina	IFAM 1313	X57204	*atpD*
Porphyromonas gingivalis	W83	Genome project[2]	*atpD* (V)
Propionigenium modestum	DSM 2376	X58461	*atpD*
Pseudomonas aeruginosa	PAO1	Genome project[2]	*atpD*
Rhodobacter capsulatus	B100	X99599	*atpD*
Rhodospirillum rubrum		X02499	*atpD*
Rickettsia prowazekii	F-12	AF036246	*atpD*
Ruminococcus albus	7ATCC	AB006151	*atpD*
Salmonella choleraesuis subsp. *arizonae*	S83769	AF037146	*atpD*
Salmonella choleraesuis subsp. *arizonae*	u24	AF037147	*atpD*
Salmonella bongori	JEO4162	AF037155	*atpD*
Salmonella bongori	BR1859	AF037154	*atpD*
Salmonella choleraesuis subsp. *diarizonae*	DS210/89	AF037149	*atpD*
Salmonella choleraesuis subsp. *diarizonae*	JEO307	AF037148	*atpD*
Salmonella choleraesuis subsp. *diarizonae*	S109671	AF037150	*atpD*
Salmonella choleraesuis subsp. *choleraesuis* serotype *dublin*	K228	AF037140	*atpD*
Salmonella choleraesuis subsp. *choleraesuis* serotype *dublin*	K771	AF037139	*atpD*
Salmonella choleraesuis subsp. *houtenae*	S84366	AF037151	*atpD*
Salmonella choleraesuis subsp. *houtenae*	S84098	AF037152	*atpD*
Salmonella choleraesuis subsp. *indica*	BR2047	AF037153	*atpD*
Salmonella choleraesuis subsp. *choleraesuis* serotype *infantis*	Div36-86	AF037142	*atpD*
Salmonella choleraesuis subsp. *salamae*	NSC72	AF037144	*atpD*
Salmonella choleraesuis subsp. *salamae*	S114655	AF037145	*atpD*
Salmonella choleraesuis subsp. *choleraesuis* serotype *tennessee*	Div95-86	AF037143	*atpD*
Salmonella choleraesuis subsp. *choleraesuis* serotype *typhimurium*	LT2	AF037141	*atpD*
Shewanella putida		Genome project[2]	*atpD*
Shewanella putrefaciens	MR-1	Genome project[2]	*atpD*
Stigmatella aurantiaca	Sga1	X76879	*atpD*
Streptococcus bovis	JB-1	AB009314	*atpD*

Table 11. **Microbial species for which *atpD* and/or *tuf* and/or *recA* and/or *Rad51* and/or *dmc1* sequences are available in public databases (continued)**

Species	Strain	Accession number	Coding gene*
Streptococcus mutans	GS-5	U31170	*atpD*
Streptococcus mutans	UAB159	Genome project[2]	*atpD*
Streptococcus pneumoniae	type 4	TIGR[2]	*atpD*
Streptococcus pneumoniae	Type 4	Genome project[2]	*atpD* (V)
Streptococcus pyogenes	M1-GAS	Genome project[2]	*atpD*
Streptococcus pyogenes		Genome project[2]	*atpD* (V)
Streptococcus sanguis	10904	AF001955	*atpD*
Streptomyces lividans	1326	Z22606	*atpD*
Thermus thermophilus	HB8	D63799	*atpD* (V)
Thiobacillus ferrooxidans	ATCC 33020	M81087	*atpD*
Treponema pallidum	Nichols	AE001228	*atpD* (V)
Vibrio alginolyticus		X16050	*atpD*
Vibrio cholerae	N16961	Genome project[2]	*atpD*
Wolinella succinogenes	DSM 1470	X76880	*atpD*
Yersinia enterocolitica	NCTC 10460	AF037157	*atpD*
Yersinia pestis	CO-92	Genome project[2]	*atpD*
Fungi			
Candida tropicalis		M64984	*atpD* (V)
Kluyveromyces lactis	2359/152	U37764	*atpD*
Neurospora crassa		X53720	*atpD*
Saccharomyces cerevisiae		M12082	*atpD*
Saccharomyces cerevisiae	X2180-1A	J05409	*atpD* (V)
Schizosaccharomyces pombe	972 h-	S47814	*atpD* (V)
Schizosaccharomyces pombe	972 h-	M57956	*atpD*
Parasites			
Giardia lamblia	WB	U18938	*atpD*
Plasmodium falciparum	3D7	L08200	*atpD* (V)
Trypanosoma congolense	IL3000	Z25814	*atpD* (V)
Human and plants			
Arabidopsis thaliana	Columbia	X89227	*tuf* (ef-1)
Glycine max	Ceresia	X89058	*tuf* (ef-1)
Glycine max	Ceresia	Y15107	*tuf* (ef-1)
Glycine max	Ceresia	Y15108	*tuf* (ef-1)
Glycine max	Maple Arrow	X66062	*tuf* (ef-1)
Pyramimonas disomata		AB008010	*tuf*
Homo sapiens		L09234	*atpD* (V)
Homo sapiens		M27132	*atpD*
Homo sapiens		X03558	*tuf* (ef-1)

Table 11. Microbial species for which *atpD* and/or *tuf* and/or *recA* and/or *Rad51* and/or *dmc1* sequences are available in public databases (continued)

Species	Strain	Accession number	Coding gene*
Bacteria			
Acetobacter aceti		D13184	*recA*
Acetobacter aceti		S60630	*recA*
Acetobacter altoacetigenes	MH-24	E05290	*recA*
Acetobacter polyoxogenes	NBI 1028	D13183	*recA*
Acidiphilium facilis	ATCC 35904	D16538	*recA*
Acinetobacter calcoaceticus	BD413/ADP1	L26100	*recA*
Acholeplasma laidlawii	8195	M81465	*recA*
Aeromonas salmonicida	A449	U83688	*recA*
Agrobacterium tumefaciens	C58	L07902	*recA*
Allochromatium vinosum		AJ000677	*recA*
Anabaena variabilis	ATCC 29413	M29680	*recA*
Aquifex pyrophilus	Kol5a	L23135	*recA*
Azotobacter vinelandii		S96898	*recA*
Bacillus subtilis	PB1831	U87792	*recA*
Bacteroides fragilis		M63029	*recA*
Bifidobacterium breve	NCFB 2258	AF094756	*recA*
Blastochloris viridis	DSM 133	AF022175	*recA*
Bordetella pertussis	165	X53457	*recA*
Borrelia burgdorferi	Sh-2-82	U23457	*recA*
Brevibacterium flavum	MJ-233	E10390	*recA*
Brucella abortus	2308	L00679	*recA*
Burkholderia cepacia	ATCC 17616	U70431	*recA*
Campylobacter jejuni	81-176	U03121	*recA*
Chlamydia trachomatis	L2	U16739	*recA*
Chloroflexus aurantiacus	J-10-fl	AF037259	*recA*
Clostridium perfringens	13	U61497	*recA*
Corynebacterium glutamicum	AS019	U14965	*recA*
Corynebacterium glutamicum	AS019	X77384	*recA*
Corynebacterium pseudotuberculosis	C231	U30387	*recA*
Deinococcus radiodurans	KD8301	AB005471	*recA*
Enterobacter agglomerans	339	L03291	*recA*
Enterococcus faecalis	OGIX	M81466	*recA*
Erwinia carotovora		X55554	*recA*
Escherichia coli		J01672	*recA*
Escherichia coli		X55552	*recA*
Escherichia coli	K-12	AE000354	*recA*
Frankia alni	Arl3	AJ006707	*recA*
Gluconobacter oxydans		U21001	*recA*
Haemophilus influenzae	Rd	U32687	*recA*
Haemophilus influenzae	Rd	U32741	*recA*
Haemophilus influenzae	Rd	L07529	*recA*
Helicobacter pylori	69A	Z35478	*recA*
Lactococcus lactis	ML3	M88106	*recA*
Legionella pneumophila		X55453	*recA*
Leptospira biflexa	serovar patoc	U32625	*recA*
Leptospira interrogans	serovar pomona	U29169	*recA*
Magnetospirillum magnetotacticum	MS-1	X17371	*recA*
Methylobacillus flagellatum	MFK1	M35325	*recA*

Table 11. Microbial species for which *atpD* and/or *tuf* and/or *recA* and/or *Rad51* and/or *dmc1* sequences are available in public databases (continued)

Species	Strain	Accession number	Coding gene*
Methylomonas clara	ATCC 31226	X59514	*recA*
Mycobacterium leprae		X73822	*recA*
Mycobacterium tuberculosis	H37Rv	X58485	*recA*
Mycoplasma genitalium	G37	U39717	*recA*
Mycoplasma mycoides	GM9	L22073	*recA*
Mycoplasma pulmonis	KD735	L22074	*recA*
Myxococcus xanthus		L40368	*recA*
Neisseria animalis	NCTC 10212	U57910	*recA*
Neisseria cinerea	LCDC 81-176	AJ223869	*recA*
Neisseria cinerea	LNP 1646	U57906	*recA*
Neisseria cinerea	NCTC 10294	AJ223871	*recA*
Neisseria cinerea	Vedros M601	AJ223870	*recA*
Neisseria elongata	CCUG 2131	AJ223882	*recA*
Neisseria elongata	CCUG 4165A	AJ223880	*recA*
Neisseria elongata subsp. *intermedia*	CCUG 4557	AJ223879	*recA*
Neisseria elongata	NCTC 10660	AJ223881	*recA*
Neisseria elongata	NCTC 11050	AJ223878	*recA*
Neisseria elongata	NHITCC 2376	AJ223877	*recA*
Neisseria flava	Bangor 9	AJ223873	*recA*
Neisseria flavescens	LNP 444	U57907	*recA*
Neisseria gonorrhoeae	CH95	U57902	*recA*
Neisseria gonorrhoeae	FA19	X64842	*recA*
Neisseria gonorrhoeae	MS11	X17374	*recA*
Neisseria lactamica	CCUC 7757	AJ223866	*recA*
Neisseria lactamica	CCUG 7852	Y11819	*recA*
Neisseria lactamica	LCDC 77-143	Y11818	*recA*
Neisseria lactamica	LCDC 80-111	AJ223864	*recA*
Neisseria lactamica	LCDC 845	AJ223865	*recA*
Neisseria lactamica	NCTC 10617	U57905	*recA*
Neisseria lactamica	NCTC 10618	AJ223863	*recA*
Neisseria meningitidis	44/46	X64849	*recA*
Neisseria meningitidis	Bangor 13	AJ223868	*recA*
Neisseria meningitidis	HF116	X64848	*recA*
Neisseria meningitidis	HF130	X64844	*recA*
Neisseria meningitidis	HF46	X64847	*recA*
Neisseria meningitidis	M470	X64850	*recA*
Neisseria meningitidis	N94II	X64846	*recA*
Neisseria meningitidis	NCTC 8249	AJ223867	*recA*
Neisseria meningitidis	P63	X64845	*recA*
Neisseria meningitidis	S3446	U57903	*recA*
Neisseria meningitidis	S3446	X64843	*recA*
Neisseria mucosa	LNP 405	U57908	*recA*
Neisseria mucosa	Vedros M1801	AJ223875	*recA*
Neisseria perflava	CCUG 17915	AJ223876	*recA*
Neisseria perflava	LCDC 85402	AJ223862	*recA*
Neisseria pharyngis	NCTC 4590	U57909	*recA*
Neisseria polysaccharea	CCUG 18031	Y11815	*recA*
Neisseria polysaccharea	CCUG 24845	Y11816	*recA*
Neisseria polysaccharea	CCUG 24846	Y11814	*recA*

Table 11. Microbial species for which *atpD* and/or *tuf* and/or *recA* and/or *Rad51* and/or *dmc1* sequences are available in public databases (continued)

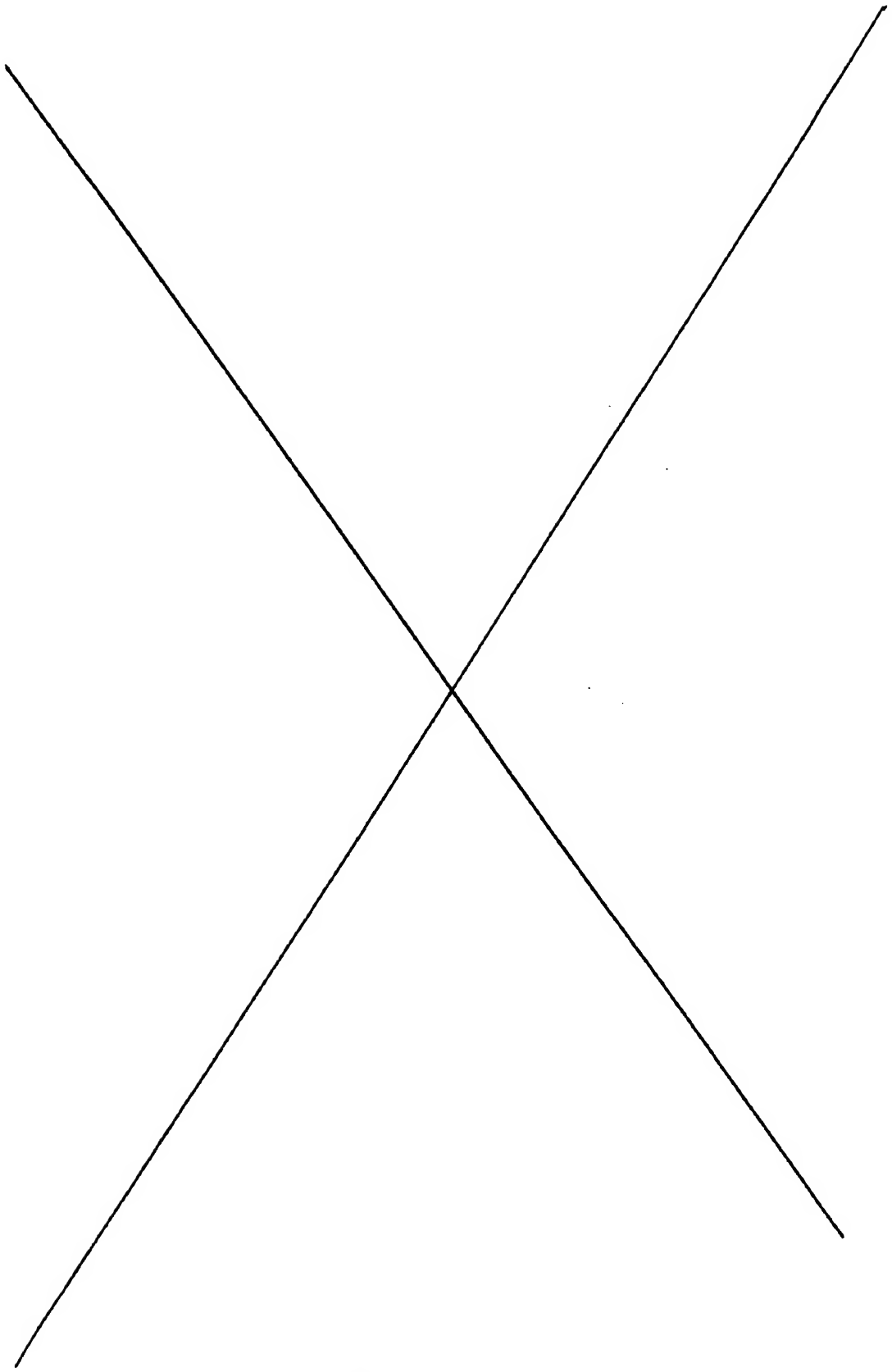
Species	Strain	Accession number	Coding gene*
Neisseria polysaccharea	INS MA 3008	Y11817	*recA*
Neisseria polysaccharea	NCTC 11858	U57904	*recA*
Neisseria sicca	NRL 30016	AJ223872	*recA*
Neisseria subflava	NRL 30017	AJ223874	*recA*
Paracoccus denitrificans	DSM 413	U59631	*recA*
Pasteurella multocida		X99324	*recA*
Porphyromonas gingivalis	W83	U70054	*recA*
Prevotella ruminicola	JCM 8958	U61227	*recA*
Proteus mirabilis	pG1300	X14870	*recA*
Proteus vulgaris		X55555	*recA*
Pseudomonas aeruginosa		X05691	*recA*
Pseudomonas aeruginosa	PAM 7	X52261	*recA*
Pseudomonas aeruginosa	PAO12	D13090	*recA*
Pseudomonas cepacia		D90120	*recA*
Pseudomonas fluorescens	OE 28.3	M96558	*recA*
Pseudomonas putida		L12684	*recA*
Pseudomonas putida	PpS145	U70864	*recA*
Rickettsia prowazekii	Madrid E	AJ235273	*recA*
Rickettsia prowazekii	Madrid E	U01959	*recA*
Rhizobium leguminosarum biovar *viciae*	*VF39*	*X59956*	*recA*
Rhizobium phaseoli	CNPAF512	X62479	*recA*
Rhodobacter capsulatus	J50	X82183	*recA*
Rhodobacter sphaeroides	2.4.1	X72705	*recA*
Serratia marcescens		M22935	*recA*
Sinorhizobium meliloti	2011	X59957	*recA*
Shigella flexneri		X55553	*recA*
Shigella sonnei	KNIH104S	AF101227	*recA*
Staphylococcus aureus		L25893	*recA*
Streptococcus gordonii	Challis V288	L20574	*recA*
Streptococcus mutans	UA96	M81468	*recA*
Streptococcus pneumoniae		Z17307	*recA*
Streptococcus pneumoniae	R800	Z34303	*recA*
Streptococcus pyogenes	NZ131	U21934	*recA*
Streptococcus salivarius subsp. *thermophilus*		M94062	*recA*
Streptomyces ambofaciens	DSM 40697	Z30324	*recA*
Streptomyces coelicolor	A3(2)	AL020958	*recA*
Streptomyces lividans	TK24	X76076	*recA*
Streptomyces rimosus	R6	X94233	*recA*
Streptomyces venezuelae	ATCC10712	U04837	*recA*
Synechococcus spp.	PR6	M29495	*recA*
Thermotoga maritima		L23425	*recA*
Thermus aquaticus		L20095	*recA*
Thermus thermophilus	HB8	D17392	*recA*
Thiobacillus ferrooxidans		M26933	*recA*
Vibrio anguillarum		M80525	*recA*
Vibrio cholerae	017	X71969	*recA*
Vibrio cholerae	2740-80	U10162	*recA*
Vibrio cholerae	569B	L42384	*recA*

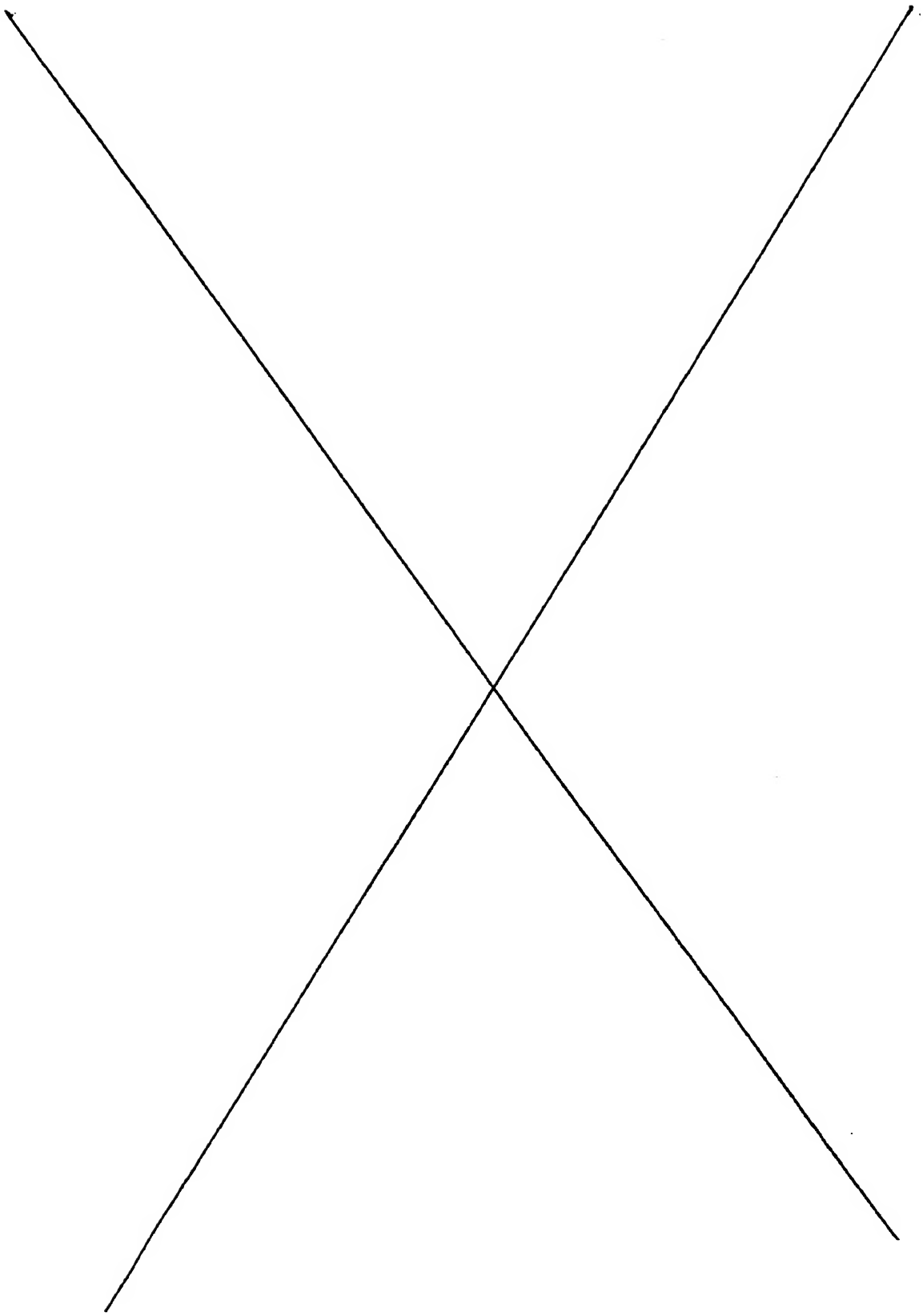
Table 11. Microbial species for which *atpD* and/or *tuf* and/or *recA* and/or *Rad51* and/or *dmc1* sequences are available in public databases (continued)

Species	Strain	Accession number	Coding gene*
Vibrio cholerae	M549	AF117881	*recA*
Vibrio cholerae	M553	AF117882	*recA*
Vibrio cholerae	M645	AF117883	*recA*
Vibrio cholerae	M793	AF117878	*recA*
Vibrio cholerae	M794	AF117880	*recA*
Vibrio cholerae	M967	AF117879	*recA*
Xanthomonas citri	XW47	AF006590	*recA*
Xanthomonas oryzae		AF013600	*recA*
Xenorhabdus bovieni	T228/1	U87924	*recA*
Xenorhabdus nematophilus	AN6	AF127333	*recA*
Yersinia pestis	231	X75336	*recA*
Fungi, parasites, human and plants			
Arabidopsis thaliana		U43652	*Rad51*
Coprinus cinereus	Okayama-7	U21905	*Rad51*
Emericella nidulans		Z80341	*Rad51*
Gallus gallus		L09655	*Rad51*
Homo sapiens		D13804	*Rad51*
Leishmania major	Friedlin	AF062379	*Rad51*
Neurospora crassa	74-OR23-1A	D29638	*Rad51*
Saccharomyces cerevisiae		D10023	*Rad51*
Schizosaccharomyces pombe		Z22691	*Rad51*
Tetrahymena thermophila	PB9R	AF064516	*Rad51*
Trypanosoma brucei	stock 427	Y13144	*Rad51*
Ustilago maydis		U62484	*Rad51*
Xenopus laevis		D38488	*Rad51*
Xenopus laevis		D38489	*Rad51*
Candida albicans		U39808	*dmc1*
Homo sapiens		D63882	*dmc1*
Leishmania major	Friedlin	AF062380	*dmc1*
Mus musculus		D58419	*dmc1*
Schizosaccharomyces pombe	972h-	AL021817	*dmc1*

* *atpD* indicates *atpD* sequences ot the F-type
atpD (V) indicates *atpD* sequences of the V-Type
tuf indicates *tuf* sequences
tuf (C) indicates *tuf* sequences divergent from main (usually A and B) copies of the elongation factor-Tu
tuf (ef-1) indicates *tuf* sequences of the eukaryotic type (elongation factor 1α)
tuf (M) indicates *tuf* sequences from organellar (mostly mitochondrial) origin
recA indicates *recA* sequences, *Rad51* indicates *Rad51* sequences or *rad51* homologs and *dmc1* indicates *dmc1* sequences or *dmc1* homologs

[1] Nucleotides sequences published in Arch. Microbiol. 1990 **153**:241-247
[2] These sequences are from TIGR database (http://www.tigr.org/tdb/tdb.html)
[3] Nucleotides sequences published in FEMS Microbiology Letters 1988 **50**:101-106





Annex I: Specific and ubiquitous primers for DNA amplification (*tuf* sequences)

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment: SEQ ID NO.	Originating DNA fragment: Nucleotide position
Bacterial species: ***Chlamydia pneumoniae***			
630	5'-CGG AGC TAT CCT AGT CGT TTC A	20	2-23
629[a]	5'-AAG TTC CAT CTC AAC AAG GTC AAT A	20	146-170
Bacterial species: ***Chlamydia trachomatis***			
554	5'-GTT CCT TAC ATC GTT GTT TTT CTC	22	82-105
555[a]	5'-TCT CGA ACT TTC TCT ATG TAT GCA	22	249-272
Parasitical species: ***Cryptosporidium parvum***			
798	5'-TGG TTG TCC CAG CCG ATC GTT T	865	158-179
804[a]	5'-CCT GGG ACG GCC TCT GGC AT	865	664-683
799	5'-ACC TGT GAA TAC AAG CAA TCT	865	280-300
805[a]	5'-CTC TTG TCC ATC TTA GCA GT	865	895-914
800	5'-GAT GAA ATC TTC AAC GAA GTT GAT	865	307-330
806[a]	5'-AGC ATC ACC AGA CTT GAT AAG	865	946-966
801	5'-ACA ACA CCG AGA AGA TCC CA	865	353-372
803[a]	5'-ACT TCA GTG GTA ACA CCA GC	865	616-635
802	5'-TTG CCA TTT CTG GTT TCG TT	865	377-396
807[a]	5'-AAA GTG GCT TCA AAG GTT GC	865	981-1000
Bacterial species: ***Neisseria gonorrhoeae***			
551	5'-GAA GAA AAA ATC TTC GAA CTG GCT A	126	256-280
552[a]	5'-TAC ACG GCC GGT GAC TAC G	126	378-396
Bacterial species: ***Streptococcus agalactiae***			
549	5'-GAA CGT GAT ACT GAC AAA CCT TTA	207-210[b]	308-331[c]
550[a]	5'-GAA GAA GAA CAC CAA CGT TG	207-210[b]	520-539[c]

[a] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[b] These sequences were aligned to derive the corresponding primer.

[c] The nucleotide positions refer to the *S. agalactiae tuf* sequence fragment (SEQ ID NO. 209).

Annex I: Specific and ubiquitous primers for DNA amplification (*tuf* sequences) (continued)

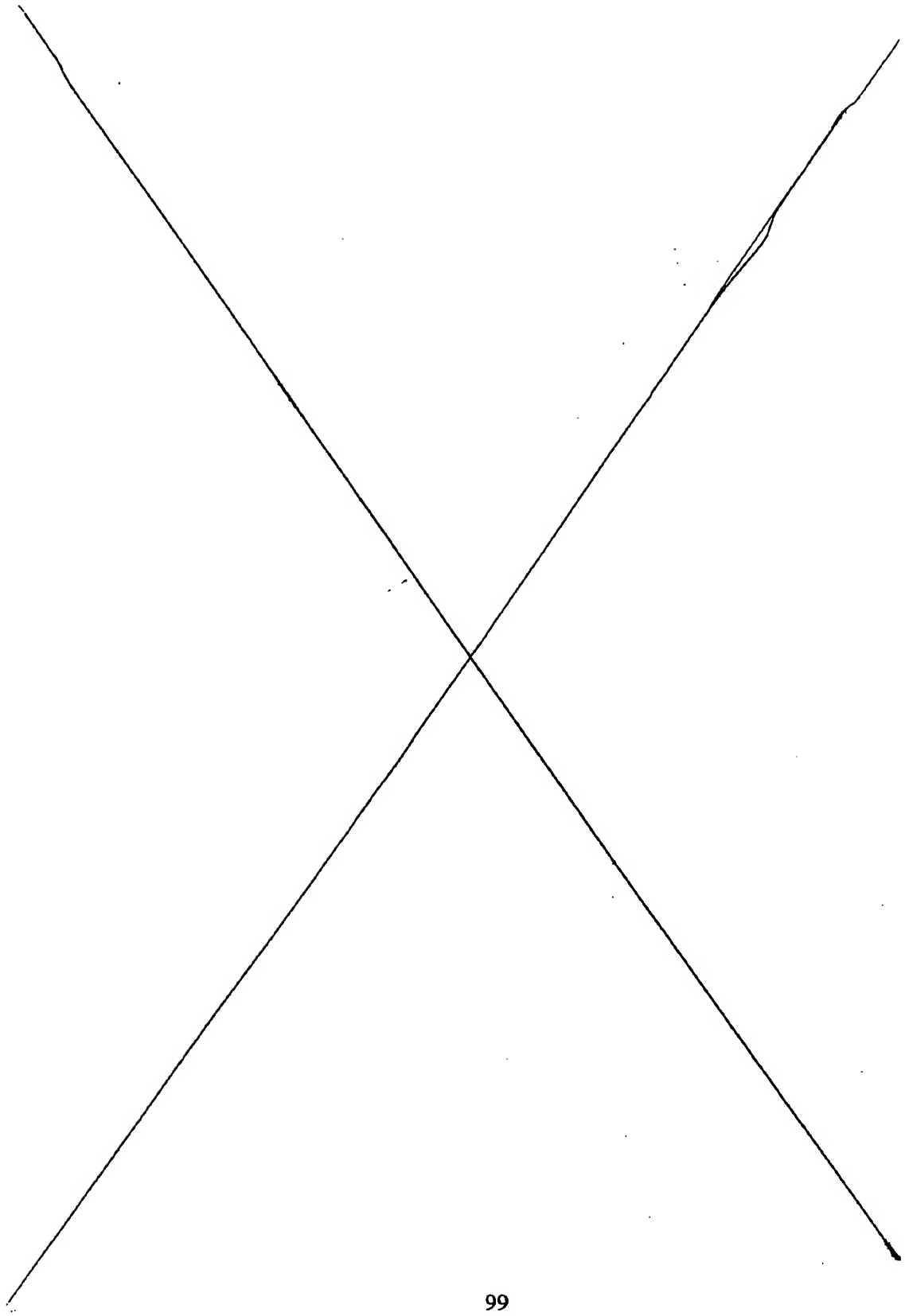
SEQ ID NO.	Nucleotide sequence	Originating DNA fragment: SEQ ID NO.	Originating DNA fragment: Nucleotide position
Parasitical species: ***Trypanosoma brucei***			
820	5'-GAA GGA GGT GTC TGC TTA CAC	864	513-533
821[a]	5'-GGC GCA AAC GTC ACC ACA TCA	864	789-809
820	5'-GAA GGA GGT GTC TGC TTA CAC	864	513-533
822[a]	5'-CGG CGG ATG TCC TTA ACA GAA	864	909-929
Parasitical species: ***Trypanosoma cruzi***			
794	5'-GAC GAC AAG TCG GTG AAC TT	840-842[b]	281-300[c]
795[a]	5'-ACT TGC ACG CGA TGT GGC AG	840-842[b]	874-893[c]
Bacterial genus: ***Bordetella spp.***			
825	5'-ATG AGC ARC GSA ACC ATC GTT CAG TG	863	1-26
826	5'-TCG ATC GTG CCG ACC ATG TAG AAC GC	863	1342-1367
Fungal genus: ***Candida spp.***			
576	5'-AAC TTC RTC AAG AAG GTY GGT TAC AA	407-426, 428-432[b]	332-357[d]
632[a]	5'-CCC TTT GGT GGR TCS TKC TTG GA	407-426, 428-432[b]	791-813[d]
631	5'-CAG ACC AAC YGA IAA RCC ATT RAG AT	407-426, 428-432[b]	523-548[d]
632[a]	5'-CCC TTT GGT GGR TCS TKC TTG GA	407-426, 428-432[b]	791-813[d]
633	5'-CAG ACC AAC YGA IAA RCC ITT RAG AT	407-426, 428-432[b]	523-548[d]
632[a]	5'-CCC TTT GGT GGR TCS TKC TTG GA	407-426, 428-432[b]	791-813[d]

[a] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[b] These sequences were aligned to derive the corresponding primer.

[c] The nucleotide positions refer to the *T. cruzi tuf* sequence fragment (SEQ ID NO. 842).

[d] The nucleotide positions refer to the *C. albicans tuf*(ef-1) sequence fragment (SEQ ID NO. 408).



Annex I: Specific and ubiquitous primers for DNA amplification (*tuf* sequences) (continued)

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment: SEQ ID NO.	Originating DNA fragment: Nucleotide position
Bacterial genus:	***Clostridium* spp.**		
796	5'-GGT CCA ATG CCW CAA ACW AGA	32,719-724,736[a]	32-52[b]
797[c]	5'-CAT TAA GAA TGG YTT ATC TGT SKC TCT	32,719-724,736[a]	320-346[b]
808	5'-GCI TTA IWR GCA TTA GAA RAY CCA	32,719-724,736[a]	224-247[b]
809[c]	5'-TCT TCC TGT WGC AAC TGT TCC TCT	32,719-724,736[a]	337-360[b]
810	5'-AGA GMW ACA GAT AAR SCA TTC TTA	32,719-724,736[a]	320-343[b]
811[c]	5'-TRA ART AGA ATT GTG GTC TRT ATC C	32,719-724,736[a]	686-710[b]
Bacterial genus:	***Corynebacterium* spp.**		
545	5'-TAC ATC CTB GTY GCI CTI AAC AAG TG	34-44,662[a]	89-114[d]
546[c]	5'-CCR CGI CCG GTR ATG GTG AAG AT	34-44,662[a]	350-372[d]
Parasitical genus:	***Entamoeba* spp.**		
703	5'-TAT GGA AAT TCG AAA CAT CT	512	38-57
704[c]	5'-AGT GCT CCA ATT AAT GTT GG	512	442-461
703	5'-TAT GGA AAT TCG AAA CAT CT	512	38-57
705[c]	5'-GTA CAG TTC CAA TAC CTG AA	512	534-553
703	5'-TAT GGA AAT TCG AAA CAT CT	512	38-57
706[c]	5'-TGA AAT CTT CAC ATC CAA CA	512	768-787
793	5'-TTA TTG TTG CTG CTG GTA CT	512	149-168
704[c]	5'-AGT GCT CCA ATT AAT GTT GG	512	442-461

[a] These sequences were aligned to derive the corresponding primer.

[b] The nucleotide positions refer to the *C. perfringens* tuf sequence fragment (SEQ ID NO. 32).

[c] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[d] The nucleotide positions refer to the *C. diphtheriae* tuf sequence fragment (SEQ ID NO. 662).

Annex I: Specific and ubiquitous prim rs for DNA amplification (*tuf* sequences) (continued)

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment: SEQ ID NO.	Originating DNA fragment: Nucleotide position
Parasitical genus:	***Trypanosoma* spp.**		
823	5'-GAG CGG TAT GAY GAG ATT GT	529,840-842,864[a]	493-512[b]
824[c]	5'-GGC TTC TGC GGC ACC ATG CG	529,840-842,864[a]	1171-1190[b]
Bacterial family:	***Mycobacteriaceae***		
539	5'-CCI TAC ATC CTB GTY GCI CTI AAC AAG	122	85-111
540[c]	5'-GGD GCI TCY TCR TCG WAI TCC TG	122	181-203
Bacterial group:	***Enterobacteriaceae* group**		
933	5'-CAT CAT CGT ITT CMT GAA CAA RTG	78,103,146, 168,238,698[a]	390-413[d]
934[c]	5'-TCA CGY TTR RTA CCA CGC AGI AGA	78,103,146, 168,238,698[a]	831-854[d]
Parasitical group:	***Kinetoplastidae* group**		
923	5'-GAC GCI GCC ATC CTG ATG ATC	511,514-526, 529,840-842, 864[a]	166-188[e]
924[c]	5'-ACC TCA GTC GTC ACG TTG GCG	511,514-526, 529,840-842, 864[a]	648-668[e]
925	5'-AAG CAG ATG GTT GTG TGC TG	511,514-526, 529,840-842, 864[a]	274-293[e]
926[c]	5'-CAG CTG CTC GTG GTG CAT CTC GAT	511,514-526, 529,840-842, 864[a]	676-699[e]

[a] These sequences were aligned to derive the corresponding primer.

[b] The nucleotide positions refer to the *T. brucei tuf* sequence fragment (SEQ ID NO. 864).

[c] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[d] The nucleotide positions refer to the *E. coli tuf* sequence fragment (SEQ ID NO. 698).

[e] The nucleotide positions refer to the *L. tropica tuf* sequence fragment (SEQ ID NO. 526).

Annex I: Specific and ubiquitous primers for DNA amplification (*tuf* sequences) (continued)

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment: SEQ ID NO.	Originating DNA fragment: Nucleotide position
Parasitical group:	***Kinetoplastidae* group (continued)**		
927	5'-ACG CGG AGA AGG TGC GCT T	511,514-526, 529,840-842, 864[a]	389-407[b]
928[c]	5'-GGT CGT TCT TCG AGT CAC CGC A	511,514-526, 529,840-842, 864[a]	778-799[b]
Bacterial group:	***Pseudomonads* group**		
541	5'-GTK GAA ATG TTC CGC AAG CTG CT	153-155[a]	476-498[d]
542[c]	5'-CGG AAR TAG AAC TGS GGA CGG TAG	153-155[a]	679-702[d]
541	5'-GTK GAA ATG TTC CGC AAG CTG CT	153-155[a]	476-498[d]
544[c]	5'-AYG TTG TCG CCM GGC ATT MCC AT	153-155[a]	749-771[d]
	Universal primers		
636	5'-ACT GGY GTT GAI ATG TTC CGY AA	7,54,78, 100,103,159, 209,224,227[b]	470-492[e]
637[a]	5'-ACG TCA GTI GTA CGG AAR TAG AA	7,54,78, 100,103,159, 209,224,227[b]	692-714[e]
638	5'-CCA ATG CCA CAA ACI CGT GAR CAC AT	7,54,78, 100,103,159, 209,224,227[b]	35-60[f]
639[a]	5'-TTT ACG GAA CAT TTC WAC ACC WGT IAC A	7,54,78, 100,103,159, 209,224,227[b]	469-496[f]

[a] These sequences were aligned to derive the corresponding primer.

[b] The nucleotide positions refer to the *L. tropica tuf* sequence fragment (SEQ ID NO. 526).

[c] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[d] The nucleotide positions refer to the *P. aeruginosa tuf* sequence fragment (SEQ ID NO. 153).

[e] The nucleotide positions refer to the *E. coli tuf* sequence fragment (SEQ ID NO. 78).

[f] The nucleotide positions refer to the *B. cereus tuf* sequenc fragment (SEQ ID NO. 7).

Annex I: Specific and ubiquitous primers for DNA amplification (*tuf* sequences) (continu d)

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
643	5'-ACT GGI GTI GAR ATG TTC CGY AA	1,3,4,7,12, 13,16,49,54, 72,78,85,88, 91,94,98,103, 108,112,115, 116,120,121, 126,128,134, 136,146,154, 159,179,186, 205,209,212, 224,238[a]	470-492[b]
644[c]	5'-ACG TCI GTI GTI CKG AAR TAG AA	1,3,4,7,12, 13,16,49,54, 72,78,85,88, 91,94,98,103, 108,112,115, 116,120,121, 126,128,134, 136,146,154, 159,179,186, 205,209,212, 224,238[a]	692-714[b]

[a] These sequences were aligned to derive the corresponding primer.

[b] The nucleotide positions refer to the *E. coli tuf* sequence fragment (SEQ ID NO. 78).

[c] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Ann x I: Specific and ubiquitous primers for DNA amplification (*tuf* sequences) (continued)

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
643	5'-ACT GGI GTI GAR ATG TTC CGY AA	1,3,4,7,12, 13,16,49,54, 72,78,85,88, 91,94,98,103, 108,112,115, 116,120,121, 126,128,134, 136,146,154, 159,179,186, 205,209,212, 224,238[a]	470-492[b]
645[c]	5'-ACG TCI GTI GTI CKG AAR TAR AA	1,3,4,7,12, 13,16,49,54, 72,78,85,88, 91,94,98,103, 108,112,115, 116,120,121, 126,128,134, 136,146,154, 159,179,186, 205,209,212, 224,238[a]	692-714[b]
646	5'-ATC GAC AAG CCI TTC YTI ATG SC	2,13,82 122,145[a]	317-339[d]
647[c]	5'-ACG TCC GTS GTR CGG AAG TAG AAC TG	2,13,82 122,145[a]	686-711[d]
646	5'-ATC GAC AAG CCI TTC YTI ATG SC	2,13,82 122,145[a]	317-339[d]
648[c]	5'-ACG TCS GTS GTR CGG AAG TAG AAC TG	2,13,82 122,145[a]	686-711[d]

[a] These sequences were aligned to derive the corresponding primer.

[b] The nucleotide positions refer to the *E. coli tuf* sequence fragment (SEQ ID NO. 78).

[c] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[d] The nucleotide positions refer to the *A. meyeri tuf* sequence fragment (SEQ ID NO. 2)

Annex I: Specific and ubiquitous primers for DNA amplification (*tuf* sequences) (continued)

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment: SEQ ID NO.	Originating DNA fragment: Nucleotide position
	Universal primers (continued)		
649	5'-GTC CTA TGC CTC ARA CWC GIG AGC AC	8,86,141,143[a]	33-58[b]
650[c]	5'-TTA CGG AAC ATY TCA ACA CCI GT	8,86,141,143[a]	473-495[b]
636	5'-ACT GGY GTT GAI ATG TTC CGY AA	8,86,141,143[a]	473-495[b]
651[c]	5'-TGA CGA CCA CCI TCY TCY TTY TTC A	8,86,141,143[a]	639-663[b]
	Sequencing primers		
556	5'-CGG CGC NAT CYT SGT TGT TGC	668[d]	306-326
557[c]	5'-CCM AGG CAT RAC CAT CTC GGT G	668[d]	1047-1068
694	5'-CGG CGC IAT CYT SGT TGT TGC	668[d]	306-326
557c	5'-CCM AGG CAT RAC CAT CTC GGT G	668[d]	1047-1068
664	5'-AAY ATG ATI ACI GGI GCI GCI CAR ATG GA	619[d]	604-632
652[c]	5'-CCW AYA GTI YKI CCI CCY TCY CTI ATA	619[d]	1482-1508
664	5'-AAY ATG ATI ACI GGI GCI GCI CAR ATG GA	619[d]	604-632
561[c]	5'-ACI GTI CGG CCR CCC TCA CGG AT	619[d]	1483-1505
543	5'-ATC TTA GTA GTT TCT GCT GCT GA	607	8-30
660[c]	5'-GTA GAA TTG AGG ACG GTA GTT AG	607	678-700
658	5'-GAT YTA GTC GAT GAT GAA GAA TT	621	116-138
659[c]	5'-GCT TTT TGI GTT TCW GGT TTR AT	621	443-465
658	5'-GAT YTA GTC GAT GAT GAA GAA TT	621	116-138
661[c]	5'-GTA GAA YTG TGG WCG ATA RTT RT	621	678-700
558	5'-TCI TTY AAR TAY GCI TGG GT	665[d]	157-176
559[c]	5'-CCG ACR GCR AYI GTY TGI CKC AT	665[d]	1279-1301
813	5'-AAT CYG TYG AAA TGC AYC ACG A	665[d]	687-708
559[c]	5'-CCG ACR GCR AYI GTY TGI CKC AT	665[d]	1279-1301

[a] These sequences were aligned to derive the corresponding primer.

[b] The nucleotide positions refer to the *B. distasonis tuf* sequence fragment (SEQ ID NO. 8).

[c] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[d] Sequences from data banks.

Annex I: Specific and ubiquitous primers for DNA amplification (*tuf* sequences) (continued)

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment: SEQ ID NO.	Originating DNA fragment: Nucleotide position
	Sequencing primers (continued)		
558	5'-TCI TTY AAR TAY GCI TGG GT	665[a]	157-176
815[b]	5'-TGG TGC ATY TCK ACR GAC TT	665[a]	686-705
560	5'-GAY TTC ATY AAR AAY ATG ATY AC	665[a]	289-311
559[b]	5'-CCG ACR GCR AYI GTY TGI CKC AT	665[a]	1279-1301
653	5'-GAY TTC ATI AAR AAY ATG AT	665[a]	289-308
559[b]	5'-CCG ACR GCR AYI GTY TGI CKC AT	665[a]	1279-1301
558	5'-TCI TTY AAR TAY GCI TGG GT	665[a]	157-176
655[b]	5'-CCR ATA CCI CMR ATY TTG TA	665[a]	754-773
654	5'-TAC AAR ATY KGI GGT ATY GG	665[a]	754-773
559[b]	5'-CCG ACR GCR AYI GTY TGI CKC AT	665[a]	1279-1301
696	5'-ATI GGI CAY RTI GAY CAY GGI AAR AC	698[a]	52-77
697[b]	5'-CCI ACI GTI CKI CCR CCY TCR CG	698[a]	1132-1154
911	5'-GAC GGM KKC ATG CCG CAR AC	853	22-41
914[b]	5'-GAA RAG CTG CGG RCG RTA GTG	853	700-720
912	5'-GAC GGC GKC ATG CCG CAR AC	846	20-39
914[b]	5'-GAA RAG CTG CGG RCG RTA GTG	846	692-712
913	5'-GAC GGY SYC ATG CCK CAG AC	843	251-270
915[b]	5'-AAA CGC CTG AGG RCG GTA GTT	843	905-925
916	5'-GCC GAG CTG GCC GGC TTC AG	846	422-441
561[b]	5'-ACI GTI CGG CCR CCC TCA CGG AT	619[a]	1483-1505
664	5'-AAY ATG ATI ACI GGI GCI GCI CAR ATG GA	619[a]	604-632
917[b]	5'-TCG TGC TAC CCG TYG CCG CCA T	846	593-614

[a] Sequences from data banks.

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex II: Specific and ubiquitous primers for DNA amplification (*atpD* sequences)

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment: SEQ ID NO.	Originating DNA fragment: Nucleotide position
Bacterial species:	***Streptococcus agalactiae***		
627	5'-ATT GTC TAT AAA AAT GGC GAT AAG TC	379-383[a]	42-67[b]
625[c]	5'-CGT TGA AGA CAC GAC CCA AAG TAT CC	379-383[a]	206-231[b]
628	5'-AAA ATG GCG ATA AGT CAC AAA AAG TA	379-383[a]	52-77[b]
625[c]	5'-CGT TGA AGA CAC GAC CCA AAG TAT CC	379-383[a]	206-231[b]
627	5'-ATT GTC TAT AAA AAT GGC GAT AAG TC	379-383[a]	42-67[b]
626[c]	5'-TAC CAC CTT TTA AGT AAG GTG CTA AT	379-383[a]	371-396[b]
628	5'-AAA ATG GCG ATA AGT CAC AAA AAG TA	379-383[a]	52-77[b]
626[c]	5'-TAC CAC CTT TTA AGT AAG GTG CTA AT	379-383[a]	371-396[b]
Bacterial genus:	***Candida spp.***		
634	5'-AAC ACY GTC AGR RCI ATT GCY ATG GA	460-472, 474-478[a]	101-126[d]
635[c]	5'-AAA CCR GTI ARR GCR ACT CTI GCT CT	460-472, 474-478[a]	617-642[d]

[a] These sequences were aligned to derive the corresponding primer.

[b] The nucleotide positions refer to the *S. agalactiae atpD* sequence fragment (SEQ ID NO. 380).

[c] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[d] The nucleotide positions refer to the *C. albicans atpD* sequence fragment (SEQ ID NO. 460).

Ann x II: Specific and ubiquitous primers for DNA amplification (*atpD* sequences) (continued)

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment: SEQ ID NO.	Originating DNA fragment: Nucleotide position
	Universal primers		
562	5'-CAR ATG RAY GAR CCI CCI GGI GYI MGI ATG	243,244,262, 264,280,284, 291,297,309, 311,315,317, 324,329,332, 334-336,339, 342,343,351, 356,357,364-366,370,375, 379,393[a]	528-557[b]
563[c]	5'-GGY TGR TAI CCI ACI GCI GAI GGC AT	243,244,262, 264,280,284, 291,297,309, 311,315,317, 324,329,332, 334-336,339, 342,343,351, 356,357,364-366,370,375, 379,393[a]	687-712[b]
564	5'-TAY GGI CAR ATG AAY GAR CCI CCI GGI AA	243,244,262, 264,280,284, 291,297,309, 311,315,317, 324,329,332, 334-336,339, 342,343,351, 356,357,364-366,370,375, 379,393[a]	522-550[b]
565[c]	5'-GGY TGR TAI CCI ACI GCI GAI GGD AT	243,244,262, 264,280,284, 291,297,309, 311,315,317, 324,329,332, 334-336,339, 342,343,351, 356,357,364-366,370,375, 379,393[a]	687-712[b]

[a] These sequences were aligned to derive the corresponding primer.

[b] The nucleotide positions refer to the *K. pneumoniae atpD* sequence fragment (SEQ ID NO. 317).

[c] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex II: Specific and ubiquitous primers for DNA amplification (*atpD* sequences) (continued)

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment: SEQ ID NO.	Originating DNA fragment: Nucleotide position
	Universal primers (continued)		
640	5'-TCC ATG GTI TWY GGI CAR ATG AA	248,284,315, 317,343,357, 366,370,379,393[a]	513-535[b]
641[c]	5'-TGA TAA CCW ACI GCI GAI GGC ATA CG	248,284,315, 317,343,357, 366,370,379,393[a]	684-709[b]
642	5'-GGC GTI GGI GAR CGI ACI CGT GA	248,284,315, 317,343,357, 366,370,379,393[a]	438-460[b]
641[c]	5'-TGA TAA CCW ACI GCI GAI GGC ATA CG	248,284,315, 317,343,357, 366,370,379,393[a]	684-709[b]
	Sequencing primers		
566	5'-TTY GGI GGI GCI GGI GTI GGI AAR AC	669[d]	445-470
567[c]	5'-TCR TCI GCI GGI ACR TAI AYI GCY TG	669[d]	883-908
566	5'-TTY GGI GGI GCI GGI GTI GGI AAR AC	669[d]	445-470
814	5'-GCI GGC ACG TAC ACI GCC TG	666[d]	901-920
568	5'-RTI ATI GGI GCI GTI RTI GAY GT	669[d]	25-47
567[c]	5'-TCR TCI GCI GGI ACR TAI AYI GCY TG	669[d]	883-908
570	5'-RTI RYI GGI CCI GTI RTI GAY GT	672[d]	31-53
567[c]	5'-TCR TCI GCI GGI ACR TAI AYI GCY TG	669[d]	883-908
572	5'-RTI RTI GGI SCI GTI RTI GA	669[d]	25-44
567[c]	5'-TCR TCI GCI GGI ACR TAI AYI GCY TG	669[d]	883-908
569	5'-RTI RTI GGI SCI GTI RTI GAT AT	671[d]	31-53
567[c]	5'-TCR TCI GCI GGI ACR TAI AYI GCY TG	669[d]	883-908
571	5'-RTI RTI GGI CCI GTI RTI GAT GT	670[d]	31-53
567[c]	5'-TCR TCI GCI GGI ACR TAI AYI GCY TG	669[d]	883-908

[a] These sequences were aligned to derive the corresponding primer.

[b] The nucleotide positions refer to the *K. pneumoniae atpD* sequence fragment (SEQ ID NO. 317).

[c] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[d] Sequences from data banks.

Annex II: Specific and ubiquitous primers for DNA amplification (*atpD* sequences) (continued)

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment	
		SEQ ID NO.	Nucleotide position
	Sequencing primers (continued)		
700	5'-TIR TIG AYG TCG ART TCC CTC ARG	669[a]	38-61
567[b]	5'-TCR TCI GCI GGI ACR TAI AYI GCY TG	669[a]	883-908
568	5'-RTI ATI GGI GCI GTI RTI GAY GT	669[a]	25-47
573[b]	5'-CCI CCI ACC ATR TAR AAI GC	666[a]	1465-1484
574	5'-ATI GCI ATG GAY GGI ACI GAR GG	666[a]	283-305
573[b]	5'-CCI CCI ACC ATR TAR AAI GC	666[a]	1465-1484
574	5'-ATI GCI ATG GAY GGI ACI GAR GG	666[a]	283-305
708[b]	5'-TCR TCC ATI CCI ARI ATI GCI ATI AT	666[a]	1258-1283
681	5'-GGI SSI TTY GGI ISI GGI AAR AC	685	694-716
682[b]	5'-GTI ACI GGY TCY TCR AAR TTI CCI CC	686	1177-1202
681	5'-GGI SSI TTY GGI ISI GGI AAR AC	685	694-716
683[b]	5'-GTI ACI GGI TCI SWI AWR TCI CCI CC	685	1180-1205
681	5'-GGI SSI TTY GGI ISI GGI AAR AC	685	694-716
699	5'-GTI ACI GGY TCY TYR ARR TTI CCI CC	686	1177-1202
681	5'-GGI SSI TTY GGI ISI GGI AAR AC	685	694-716
812[b]	5'-GTI ACI GGI TCY TYR ARR TTI CCI CC	685	1180-1205

[a] Sequences from data banks.

[b] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex III: Specific and ubiquitous probes for hybridization (*tuf* sequences)

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment: SEQ ID NO.	Originating DNA fragment: Nucleotide position
Bacterial species:	***Candida albicans***		
577	5'-CAT GAT TGA ACC ATC CAC CA	407-411[a]	406-425[b]
Bacterial species:	***Candida dubliniensis***		
578	5'-CAT GAT TGA AGC TTC CAC CA	412,414-415[a]	418-437[c]
Bacterial species:	***Enterococcus faecalis***		
580	5'-GCT AAA CCA GCT ACA ATC ACT CCA C	62-63,607[a]	584-608[d]
603	5'-GGT ATT AAA GAC GAA ACA TC	62-63,607[a]	440-459[d]
Bacterial species:	***Enterococcus faecium***		
602	5'-AAG TTG AAG TTG TTG GTA TT	64,608[a]	426-445[e]
Bacterial species:	***Enterococcus gallinarum***		
604	5'-GGT GAT GAA GTA GAA ATC GT	66,609[a]	419-438[f]
Bacterial species:	***Escherichia coli***		
579	5'-GAA GGC CGT GCT GGT GAG AA	78	503-522

[a] These sequences were aligned to derive the corresponding primer.

[b] The nucleotide positions refer to the *C. albicans tuf*(ef-1) sequence fragment (SEQ ID NO. 408).

[c] The nucleotide positions refer to the *C. dubliniensis tuf*(ef-1) sequence fragment (SEQ ID NO. 414).

[d] The nucleotide positions refer to the *E. faecalis tuf* sequence fragment (SEQ ID NO. 607).

[e] The nucleotide positions refer to the *E. faecium tuf* sequence fragment (SEQ ID NO. 608).

[f] The nucleotide positions refer to the *E. gallinarum tuf* sequence fragment (SEQ ID NO. 609).

Annex III: Specific and ubiquitous probes for hybridization (*tuf* sequences) (continued)

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment: SEQ ID NO.	Originating DNA fragment: Nucleotide position
Bacterial species: ***Haemophilus influenzae***			
581	5'-ACA TCG GTG CAT TAT TAC GTG G	610[a]	551-572[b]
Bacterial species: ***Staphylococcus aureus***			
584	5'-ACA TGA CAC ATC TAA AAC AA	176-180[c]	369-388[d]
585	5'-ACC ACA TAC TGA ATT CAA AG	176-180[c]	525-544[d]
586	5'-CAG AAG TAT ACG TAT TAT CA	176-180[c]	545-564[d]
587	5'-CGT ATT ATC AAA AGA CGA AG	176-180[c]	555-574[d]
588	5'-TCT TCT CAA ACT ATC GTC CA	176-180[c]	593-612[d]
Bacterial species: ***Staphylococcus epidermidis***			
589	5'-GCA CGA AAC TTC TAA AAC AA	185,611[c]	445-464[e]
590	5'-TAT ACG TAT TAT CTA AAG AT	185,611[c]	627-646[e]
591	5'-TCC TGG TTC TAT TAC ACC AC	185,611[c]	586-605[e]
592	5'-CAA AGC TGA AGT ATA CGT AT	185,611[c]	616-635[e]
593	5'-TTC ACT AAC TAT CGC CCA CA	185,611[c]	671-690[e]
Bacterial species: ***Staphylococcus haemolyticus***			
594	5'-ATT GGT ATC CAT GAC ACT TC	186,188-190[c]	437-456[f]
595	5'-TTA AAG CAG ACG TAT ACG TT	186,188-190[c]	615-634[f]
Bacterial species: ***Staphylococcus hominis***			
596	5'-GAA ATT ATT GGT ATC AAA GA	191,193-196[c]	431-450[g]
597	5'-ATT GGT ATC AAA GAA ACT TC	191,193-196[c]	437-456[g]
598	5'-AAT TAC ACC TCA CAC AAA AT	191,193-196[c]	595-614[g]

[a] Sequences from data banks.

[b] The nucleotide positions refer to the *H. influenzae tuf* sequence fragment (SEQ ID NO. 610).

[c] These sequences were aligned to derive the corresponding probe.

[d] The nucleotide positions refer to the *S. aureus tuf* sequence fragment (SEQ ID NO. 179).

[e] The nucleotide positions refer to the *S. epidermidis tuf* sequence fragment (SEQ ID NO. 611).

[f] The nucleotide positions refer to the *S. haemolyticus tuf* sequence fragment (SEQ ID NO. 186).

[g] The nucleotide positions refer to the *S. hominis tuf* sequence fragment (SEQ ID NO. 191).

Annex III: Specific and ubiquitous probes for hybridization (*tuf* sequences) (continued)

SEQ ID NO.	Nucleotide sequence	Originating DNA fragment: SEQ ID NO.	Originating DNA fragment: Nucleotide position
Bacterial species:	***Staphylococcus saprophyticus***		
599	5'-CGG TGA AGA AAT CGA AAT CA	198-200[a]	406-425[b]
600	5'-ATG CAA GAA GAA TCA AGC AA	198-200[a]	431-450[b]
601	5'-GTT TCA CGT GAT GAT GTA CA	198-200[a]	536-555[b]
695	5'-GTT TCA CGT GAT GAC GTA CA	198-200[a]	563-582[b]
Bacterial species:	***Streptococcus agalactiae***		
582[c]	5'-TTT CAA CTT CGT CGT TGA CAC GAA CAG T	207-210[a]	404-431[d]
583[c]	5'-CAA CTG CTT TTT GGA TAT CTT CTT TAA TAC CAA CG	207-210[a]	433-467[d]
Bacterial group:	***Enterococcus casseliflavus-flavescens-gallinarum group***		
620	5'-ATT GGT GCA TTG CTA CGT	58,65,66[a]	527-544[e]
Bacterial genus:	***Staphylococcus spp.***		
605	5'-GAA ATG TTC CGT AAA TTA TT	176-203[a]	403-422[f]
606	5'-ATT AGA CTA CGC TGA AGC TG	176-203[a]	420-439[f]

[a] These sequences were aligned to derive the corresponding primer.

[b] The nucleotide positions refer to the *S. saprophyticus tuf* sequence fragment (SEQ ID NO. 198).

[c] These sequences are from the complementary DNA strand of the sequence of the originating fragment given in the Sequence Listing.

[d] The nucleotide positions refer to the *S. agalactiae tuf* sequence fragment (SEQ ID NO. 209).

[e] The nucleotide positions refer to the *E. flavescens tuf* sequence fragment (SEQ ID NO. 65).

[f] The nucleotide positions refer to the *S. aureus tuf* sequence fragment (SEQ ID NO. 179).

Annex IV: Strategy for the selection of amplification/sequencing primers from *atpD* sequences.

	23 49	443 472	881 910	SEQ ID NO:
B. cepacia	AGTgCAT CGGCGCCGTT ATCGACGTGG...	TGTTCG GCGGTGCTGG CGTGGGCAAG ACCG...	TCCA GGCCGTGT ACGTCCCTGC GGACGACT	-
B. pertussis	AGTgCAT CGGCGCCGTG GTGGATATTC...	TGTTCG GCGGCGCCGG CGTGGGCAAG ACCG...	TCCA GGCCGTGT ACGTGCCTGC CGACGACT	-
P. aeruginosa	AAATCAT CGGCGCCGTG ATCGACGTGG...	TGTTCG GCGGCGCCGG CGTGGGCAAG ACCG...	TCCA GGCCGTAT ACGTTCCCGC GGACGACC	-
E. coli	AGGTAAT CGGCGCCGTA GTTGACGTCG...	TGTTCG GTGGTGCGGG TGTAGGTAAA ACCG...	TACA GGCAGTAT ACGTACCTGC GGATGACT	669
N. gonorrhoeae	AAATTAT CGGTGCGGTT GTTGACGTGG...	TGTTCG GCGGTGCCGG TGTGGGTAAA ACCG...	TCCA AGCCGTAT ATGTACCTGC GGATGACT	-
M. thermoacetica	AGGTTAT TGGCCCGGTG GTTGACGTCG...	TCTTCG GCGGCGCCGG GGTCGGCAAG ACGG...	TGCA AGCTATCT ATGTGCCGGC CGACGACC	-
S. aurantiaca	AGGTTcT CGGTCCCGTG ATTGACGTGG...	TGTTCG GCGGCGCCGG CGTGGGCAAG ACGG...	TGCA GGCCATCT ACGTGCCCGC CGACGACC	-
M. tuberculosis	GGGTCAC TGGGCCCGTC GTCGACGTCG...	TGTTCG GCGGTGCCGG GGTGGGCAAG ACGG...	TGCA AGCCGTCT ACGTGCCCGC CGACGACT	-
B. fragilis	AGGTAAT TGGCCCTGTG GTCGATGTGT...	TGTTTG GCGGGGCCGG AGTGGGTAAA ACTG...	TGCA GGCTGTTT ACGTACCGGC TGATGACT	-
C. lytica	AAATTAT TGGCCCAGTT ATAGATGTGG...	TATTTG GAGGTGCCGG AGTAGGTAAA ACAG...	TACA GGCGGTTT ACGTACCTGC GGATGATT	672
A. woodii	AGGTTAT TGGACCAGTA GTCGATGTTA...	TTTTCG GTGGTGCCGG AGTTGGTAAA ACCG...	TTCA GGCCGTTT ACGaTCCAGC CGATGACT	-
C. acetobutylicum	AGGTAAT AGGACCTGTT GTGGATATTA...	TGTTCG GTGGTGCCGG TGTTGGTAAA ACAG...	TTCA GGCTGTAT ATGTTCCTGC TGATGACC	671
M. pneumoniae	AAGTGAT TGGCCCGGTA GTTGATGTCA...	TATTTG GTGGTGCTGG TGTTGGTAAA ACGG...	TGCA AGCGATCT ATGTGCCAGC TGATGACT	-
H. pylori	AGGTTtT AGGCCCGGTG GTAGATGTGG...	TGTTTG GTGGGGCTGG CGTAGGCAAA ACGG...	TTCA AGCGGTGT ATGTGCCAGC AGACGACT	670
Selected sequence[a]	RTIAT IGGIGCIGTI RTIGAYGT			568
Selected sequence[a]	RTIRY IGGICCIGTI RTIGAYGT			570
Selected sequence[a]	RTIRT IGGISCIGTI RTIGA			572
Selected sequence[a]	RTIRT IGGISCIGTI RTIGATAT			569
Selected sequence[a]	RTIRT IGGICCIGTI RTIGATGT			571
Selected sequence[a]		TTYG GIGGIGCIGG IGTIGGIAAR AC		566
Selected sequence[a,b]			CA RGCIRTIT AYGTICCIGC IGAYGA	567

The sequence numbering refers to the *Escherichia coli atpD* gene fragment (SEQ ID NO: 669).
Nucleotides in capitals are identical to the selected sequence or match that sequence. Mismatches are indicated by lower cases.

[a] "R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

[b] This sequence is the reverse-complement of the selected universal primer SEQ ID NO: 567.

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Annex V: Strategy for the selection of universal amplification/sequencing primers from *atpD* (V-type) sequences.

	691	719	1177	1208	SEQ ID NO:
E. hirae	CC AGGTCCGTTT GGTGCAGGGA AGACAGT...		TCTGGTGGAg ATaTCtctGA ACCAGTGACT	CA	685
H. salinarum	CC GGGGCCGTTC GGGTCCGGGA AGACGGT...		CCCGGCGGGg ACTTCtccGA GCCGGTCACC	CA	687
T. thermophilus	CC TGGGCCCTTC GGCAGCGGCA AGACCGT...		CCGGGCGGCg ACaTgtccGA GCCCGTGACC	CA	693
Human	CC TGGGGCCTTC GGATGTGGCA AGACTGT...		CCCGGTGGAg ACTTCtcAGA tCCCGTGACG	AC	688
T. congolense	CC TGGCGCGTTT GGATGCGGAA AGACGGT...		CCTGGAGGTg ACTTTtctGA cCCAGTGACG	TC	692
P. falciparum	CC TGGTGCATTT GGTTGTGGAA AAACTTG...		CCAGGTGGTg ATTTCtctGA cCCTGTAACT	AC	689
C. pneumoniae	CC AGGACCTTTT GGTGCAGGGA AAACAGT...		GCAGGAGGAA ACTTTGAAGA	ACCAGTCACT CA	686
Selected sequences[a]	GGISSITTY GGIISIGGIA ARAC		GGIGGIA AYTTYGARGA GGIGGIG AYWTIWSIGA	RCCIGTIAC ICCIGTIAC	
Selected universal primers sequences :	SEQ ID NO: 681 GGISSITTY GGIISIGGIA ARAC		SEQ ID NO: 682[b] GTIACIG GYTCYTCRAA SEQ ID NO: 683[b] GTIACIG GITCISWIAW	RTTICCICC RTCICCICC	

The sequence numbering refers to the *Enterococcus hirae atpD* gene fragment (SEQ ID NO: 685). Nucleotides in capitals are identical to the selected sequence SEQ ID NO: 681 or 682 or match that sequences. Mismatches are indicated by lower cases. Mismatches for SEQ ID NO: 683 are indicated by underlined nucleotides.

[a] "R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

[b] This sequence is the reverse-complement of the above *atpD* sequence.

Annex VI: Strategy for the selection of universal amplification/sequencing primers from *tuf* (M) sequences (organelle origin).

	375	410	1254	1286	SEQ ID NO:
C. neoformans[a]	AAGAA CATGATCACC GGTaCCtCCC AGgctGACTG...		CGCcgTCcGA GAcatGcGAC AGACcGTTGc	CGT	-
S. cerevisiae[a]	AAGAA CATGATTACT GGTaCTtCTC AAgctGACTG...		CGCTgTCAGA GAcatGaGAC AAACTGTcGc	TGT	665
O. volvulus[a]	AAGAA TATGATCACA GGTaCTtCTC AGgctGACTG...		TGCTgTGcGt GAtatGaGAC AAACaGTTGc	GGT	-
Human[a]	AAAAA CATGATTACA GGGaCAtCTC AGgctGACTG...		TGCTgTTcGt GAtatGaGAC AGACaGTTGc	TGT	-
G. max B1[b]	AAGAA CATGATCACC GGCGCTGCCC AGATGGACGG...		TGCTATTAGA GAAGGAGGCA AAACTGTTGG	AGC	-
G. max B2[b]	AAAAA CATGATCACC GGCGCCGCCC AGATGGACGG...		TGCTATTAGA GAAGGAGGCA AAACTGTTGG	AGC	-
E. coli[c]	AAAAA CATGATCACC GGTGCTGCTC AGATGGACGG...		CGCaATCcGt GAAGGCGGCC GTACcGTTGG	CGC	-
S. aureofaciens[c]	AAGAA CATGATCACC GGTGCCGCCC AGATGGACGG...		CGCcATCcGt GAGGGTGGTC GTACcGTgGG	CGC	-
E. tenella[b]	AAAAA TATGATTACA GGAGCAGCAC AAATGGATGG...		TGCTATAAGA GAAGGAGGAA AAACTATAGG	AGC	-
T. gondii[b]	AAGAA TATGATTACT GGAGCCGCAC AAATGGATGG...		TGCTATTAGA GAAGGAGGTC GTACTATAGG	AGC	-
S. cerevisiae[b]	AAGAA TATGATTACC GGTGCTGCTC AAATGGATGG...		CAATATCAGA GAGGGTGGAA GAACTGTTGG	TAC	619
A. thaliana[b]	AAAAA TATGATTACT GGAGCTGCGC AAATGGATGG...		TGCctTAAGG GAAGGAGGTA GAACaGTTGG	AGC	-
Selected sequences[d]	AA YATGATIACI GGIGCIGCIC ARATGGA		TATIAGR GARGGIGGIM RIACTRTWGG ATCCGT GAGGGYGGCC GITCIGT		
Selected universal primer sequences:	SEQ ID NO: 664 AA YATGATIACI GGIGCIGCIC ARATGGA		SEQ ID NO: 652[e] CCWAYAG TIYKICCICC YTCYCTIATA SEQ ID NO: 561[e] ACIGTI CGGCCRCCCT CACGGAT		

The sequence numbering refers to the *Saccharomyces cerevisiae tuf* (M) gene fragment (SEQ ID NO: 619). Nucleotides in capitals are identical to the selected sequence SEQ ID NO: 652 or match that sequence. Mismatches are indicated by lower cases. Mismatches for SEQ ID NO: 561 are indicated by underlined nucleotides.

[a] This sequence refers to ef-1 gene.

[b] This sequence refers to *tuf* (M) or organelle gene.

[c] This sequence refers to *tuf* gene from bacteria.

[d] "R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

[e] This sequence is the reverse-complement of the above *tuf* sequence.

Annex VII:Strategy for the selection of ukaryotic s quencing primers from *tuf* (ef-1) sequences.

	154	179	286	314	SEQ ID NO:
S. cerevisiae	GG TTCTTTCAAG TACGCTTGGG	TTTT...AGAGA	TTTCATCAAG AACATGATTA	CTGG...	665
B. hominis	GG CTCCTTCAAG TACGCGTGGG	TGCT...CGTGA	CTTCATaAAG AACATGATCA	CGGG...	-
C. albicans	GG TTCTTTCAAA TACGCTTGGG	TCTT...AGAGA	TTTCATCAAG AATATGATCA	CTGG...	-
C. neoformans	TC TTCTTTCAAG TACGCTTGGG	TTCT...CGAGA	CTTCATCAAG AACATGATCA	CCGG...	-
E. histolytica	GG ATCATTCAAA TATGCTTGGG	TCTT...AGAGA	TTTCATTAAG AACATGATTA	CTGG...	-
G. lamblia	GG CTCCTTCAAG TACGCGTGGG	TCCT...CGCGA	CTTCATCAAG AACATGATCA	CGGG...	-
H. capsulatum	AA ATCCTTCAAA TATGCGTGGG	TCCT...CGTGA	CTTCATCAAG AACATGATCA	CTGG...	-
Human	GG CTCCTTCAAG TATGCCTGGG	TCTT...AGAGA	CTTtATCAAA AACATGATTA	CAGG...	-
L. braziliensis	GC GTCCTTCAAG TACGCGTGGG	TGCT...CGCGA	CTTCATCAAG AACATGATCA	CCGG...	-
O. volvulus	GG CTCATTTAAA TATGCTTGGG	TATT...CGTGA	TTTCATTAAG AATATGATCA	CAGG...	-
P. berghei	GG TagTTTCAAA TATGCATGGG	TTTT...AAAcA	TTTtATTAAA AATATGATTA	CTGG...	-
P. knowlesi	GG AagTTTTAAG TACGCATGGG	TGTT...AAGGA	TTTCATTAAA AATATGATTA	CCGG...	-
S. pombe	GG TTCCTTCAAG TACGCCTGGG	TTTT...CGTGA	TTTCATCAAG AACATGATTA	CCGG...	-
T. cruzi	TC TTCTTTCAAG TACGCGTGGG	TCTT...CGCGA	CTTCATCAAG AACATGATCA	CGGG...	-
Y. lipolytica	GG TTCTTTCAAG TACGCTTGGG	TTCT...CGAGA	TTTCATCAAG AACATGATCA	CCGG...	-
Selected sequences[a]	TCITTYAAR TAYGCITGGG T		GA YTTCATYAAR AAYATGATYA	C	
Selected sequences[a]			GA YTTCATIAAR AAYATGAT		

Selected amplification primers sequences:	SEQ ID NO: 558 TCITTYAAR TAYGCITGGG T	SEQ ID NO: 560 GA YTTCATYAAR AAYATGATYA C SEQ ID NO: 653 GA YTTCATIAAR AAYATGAT

The sequence numbering refers to the *Saccharomyces cerevisiae tuf* (ef-1) gene fragment (SEQ ID NO: 665). Nucleotides in capitals are identical to the selected sequence SEQ ID NO: 558 or 560 or match that sequence. Mismatches are indicated by lower cases. Mismatches for SEQ ID NO: 653 are indicated by underlined nucleotides.

[a] "R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

Annex VII: Strategy for the selection of eukaryotic sequencing primers from *tuf* (ef-1) sequences (continued).

	751	770	1276	1304	SEQ ID NO:
S. cerevisiae	GTTTACAA GATCGGTGGT ATTGGTAC	...	GACATG AGACAAACTG TCGCTGTCGG	TGT	665
B. hominis	GTGTACAA GATTGGCGGT ATTGGTAC	...	GATATG AGACAGACTG TCGCTGTCGG	TAT	-
C. albicans	GTTTACAA GATCGGTGGT ATTGGTAC	...	GATATG AGACAAACCG TTGCTGTtGG	TGT	-
C. neoformans	GTCTACAA GATCGGTGGT ATCGGCAC	...	GACATG CGACAGACCG TTGCCGTtGG	TGT	-
E. histolytica	GTTTACAA GATTTcAGGT ATTGGAAC	...	GATATG AaACAAACCG TTGCTGTtGG	AGT	-
G. lamblia	GTCTACAA GATCTcGGGc gTCGGGAC	...	~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~	~~~	-
H. capsulatum	GTGTACAA AATCTcTGGT ATTGGCAC	...	GACATG AGACAAACCG TCGCTGTCGG	TGT	-
Human	GTCTACAA AATTGGTGGT ATTGGTAC	...	GATATG AGACAGACAG TTGCgGTgGG	TGT	-
L. braziliensis	GTGTACAA GATCGGCGGT ATCGGCAC	...	GACATG CGCagAACGG TCGCCGTCGG	CAT	-
O. volvulus	GTTTACAA AATTGGAGGT ATTGGAAC	...	GATATG AGACAAACAG TTGCTGTtGG	CGT	-
P. berghei	GTATACAA AATTGGTGGT ATTGGTAC	...	GATATG AGACAAACAA TTGCTGTCGG	TAT	-
P. knowlesi	GTATACAA AATCGGTGGT ATTGGTAC	...	GATATG AGACAAACCA TTGCTGTCGG	TAT	-
S. pombe	GTTTACAA GATCGGTGGT ATTGGTAC	...	GACATG CGTCAAACCG TCGCTGTCGG	TGT	-
T. cruzi	GTGTACAA GATCGGCGGT ATCGGCAC	...	GACATG CGCCAGACGG TCGCCGTCGG	CAT	-
Y. lipolytica	GTCTACAA GATCGGTGGT ATCGGCAC	...	GACATG CGACAGACCG TTGCTGTCGG	TGT	-
Selected sequences[a]	TACAA RATYKGIGGT ATYGG		ATG MGICARACIR TYGCYGTCGG		
Selected amplification primers sequences:	SEQ ID NO: 654 TACAA RATYKGIGGT ATYGG		SEQ ID NO: 559[b] CCG ACRGCRAYIG TYTGICKCAT		
	SEQ ID NO: 655[b] CCRAT ACCICMRATY TTGTA				

The sequence numbering refers to the *Saccharomyces cerevisiae tuf* (ef-1) gene fragment (SEQ ID NO: 665). Nucleotides in capitals are identical to the selected sequence or match that sequence. Mismatches are indicated by lower cases. "~" indicate incomplete sequence data. Dots indicate gaps.

[a] "R" "Y" "M" "K" "W" and "S" designate nucleotide positions which are degenerated. "R" stands for A or G; "Y" stands for C or T; "M" stands for A or C; "K" stands for G or T; "W" stands for A or T; "S" stands for C or G. "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T.

[b] This sequence is the reverse-complement of the above *tuf* (ef-1) sequence.